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EVALUATION OF IMMUNOLOGICAL CHANGES IN PATIENTS WITH DIFFUSE FORM OF AUTOIMMUNE THYROIDITIS DURING LASER PHOTODYNAMIC THERAPY

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Conflict of interest

The authors declare that they have no conflicts of interest

Abstract

The purpose of the study. To study the nature and dynamics of changes in humoral and local immunity during laser photodynamic therapy in patients with diffuse forms of autoimmune thyroiditis.

Materials and methods. Laboratory tests of blood plasma were performed on 160 patients with long-lasting autoimmune thyroiditis in different age groups to determine humoral and local immunity. Here, information on the level of immunoglobulins A, G, M (IgA, IgG, IgM), the amount of interleukin-1 β (IL-1 β), tumor necrosis factor α (TNF- α) was determined in blood samples by the immunoenzyme method. The dynamics of laboratory parameters in all three groups of patients were studied on days 7 and 15 of treatment. These values were determined using reagents from "Vector-Best" LLC (Russia).

Results. In elderly patients with long-term autoimmune thyroiditis, a downward trend in TNF indices has been observed, which is an indication of the severity of the pathological process. The higher the amount of α 2-MG in autoimmune thyroiditis and diffuse toxic urination, and the slower the normalization during treatment, the higher the probability of recurrence of the process.

Conclusion. The combined use of modern laser technology in the treatment of patients with autoimmune thyroiditis expands the possibilities of conservative therapy and complements the arsenal of effective methods of treatment of this disease. The simplicity of the methods, ease of application, reliability, the absence of thermal effects on the thyroid gland creates ample opportunities for the application of this method in clinical practice.

Keywords

autoimmune thyroiditis, immunoglobulin, tumor necrosis factor α , interleukin-1 β , lactoferrin

Аутоиммунды тиреоидиттің диффузиялық түрімен ауыратын науқастарда лазерлі фотодинамиялық терапия кезіндегі иммунологиялық өзгерістерді бағалау

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Аңдатпа

Жұмыстың мақсаты - аутоиммунды тиреоидиттің диффузды түрімен ауыратын науқастарда лазерлік фотодинамиялық терапия кезінде гуморальды және жергілікті иммунитеттің, қалқанша безінің параметрлерінің өзгеру сипаты мен динамикасын зерттеу болып табылады.

Материал және әдістер. Жұмыс ұзақ мерзімді аутоиммунды тиреоидитпен ауыратын әртүрлі жас топтарындағы 160 пациенттің гуморальды және жергілікті иммунитет көрсеткіштерін анықтау деректеріне негізделген. Қан плазмасына зертханалық зерттеу жүргізілді, оның үлгілерінде иммуноглобулиндер А, G, M (IgA, IgG, IgM), интерлейкин-1 β (IL-1 β), ісік некроз факторы- α (TNF-) анықталды. а) иммундық талдау ферментімен анықталды. Осы зертханалық көрсеткіштердің динамикасы емдеудің 7 және 15-ші күндерінде барлық үш топтағы науқастарда зерттелді. Бұл көрсеткіштер «Вектор-Бест» (Ресей) компаниясының реагенттері арқылы анықталды. Материал емдеуге дейін, емдеу басталғаннан кейін 7 және 15 күннен кейін алынды.

Нәтижелер. Ұзақ мерзімді аутоиммунды тиреоидитпен (АИТ) TNF индекстерінің төмендеуіне тенденция байқалады, бұл патологиялық процестің ауырлығын көрсетеді. Аутоиммунды тиреоидит пен диффузды токсикалық зоб (ДТГ) кезінде α 2-MG мөлшері неғұрлым жоғары болса және емдеу кезінде олардың қалыпқа келуі неғұрлым баяу болса, процестің қайталану ықтималдығы соғұрлым жоғары болады деп болжанады.

Қорытынды. АИТ бар науқастарды емдеуде лазерлік технологияларды қолдану консервативті терапияның мүмкіндіктерін кеңейтеді және осы ауруды емдеудің тиімді әдістерінің арсеналын толықтырады. Әдістердің қарапайымдылығы, олардың қолжетімділігі, сенімділігі, қалқанша безінің термиялық зақымдануын болдырмау біздің терең сеніміміз бойынша бұл әдістерді клиникалық тәжірибеге енгізуге негіз береді.

Түйін сөздер

аутоиммунды тиреоидит, иммуноглобулин, ісік некрозының факторы- α , интерлейкин-1 β , лактоферрин

Оценка иммунологических изменений при проведении лазерной фотодинамической терапии у больных диффузной формой аутоиммунного тиреоидита

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Аннотация

Цель работы является изучить характер и динамику изменений показателей гуморального и местного иммунитета, щитовидной железы при проведении лазерной фотодинамической терапии у больных диффузной формой аутоиммунного тиреоидита.

Материал и методы. Работа основана на данных определения показателей гуморального и местного иммунитета 160 больных разных возрастных групп с длительно текущим аутоиммунным тиреоидитом. Выполнено лабораторное исследование плазмы крови, в образцах которых иммуноферментным методом определяли содержание иммуноглобулинов А, G, М (IgA, IgG, IgM), интерлейкина-1 β (ИЛ-1 β), фактора некроза опухоли- α (ФНО- α). У пациентов всех трех групп изучена динамика данных лабораторных показателей на 7-й и 15-й день лечения. Данные показатели определяли с использованием реактивов компании ООО «Вектор-Бест» (Россия). Забор материала осуществляли до лечения, через 7 и 15 суток от начала лечения.

Результаты. При длительно текущем аутоиммунном тиреоидите (АИТ) отмечается тенденция снижения показателей ФНО, что указывает на выраженность патологического процесса. Предполагается, что чем выше содержание а2-МГ при аутоиммунном тиреоидите и диффузно-токсическом зобе (ДТЗ), и чем медленнее их нормализация при лечении, тем выше вероятность возникновения рецидива процесса.

Заключение. Применение лазерных технологий в лечении больных АИТ расширяет возможности консервативной терапии и дополняет арсенал эффективных методов лечения этого заболевания. Простота способов, их доступность, надежность, исключение термического повреждения щитовидной железы дают, по нашему глубокому убеждению, основание к внедрению этих методов в клиническую практику.

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Ключевые слова

аутоиммунный тиреоидит, иммуноглобулин, фактора некроза опухоли- α , интерлейкина-1 β , лактоферрин

Introduction

Autoimmune thyroiditis is a typical autoimmune disease characterized by chronic inflammation of the thyroid gland. A characteristic morphological sign of autoimmune thyroiditis (AIT) is lymphoplasmacytic infiltration of the thyroid tissue of the gland, with obligatory autoimmune inflammation. The composition of cells in AIT is always constant; it combines cells of the lymphoid series, plasmacytic infiltration, and macrophages. An increased level of antibodies to the thyroid gland in the blood leads to an increase in the functional activity of mononuclear cells, which destroy thyrocytes and follicles with the release of cytokines and lactoferrin (Lf). Cytokines play an important role in modulating immune responses and initiating the immune process. Their role is often difficult to predict without taking into account other mediators - immune and hormonal. Regulatory cytokines can induce self-tolerance to thyroid antigens or, on the contrary, activate autoimmune processes in it. An excess of Lf in the blood does not improve the situation; on the contrary, it supports inflammatory and autoimmune changes, increases the production of TNF- α and lactoferrin (Lf) cytokines in the blood serum of AIT patients [1,4,12].

Meanwhile, in practical medical institutions, clinical diagnosis is often formulated as a nodular (or multinodular) goiter. However, from a practical point of view, identifying the main forms of

thyroiditis is extremely important, since applying an accurate classification will vividly establish the nature (with/without complications) of the clinical course of the disease, while also enabling us to objectively assess the prognosis of the disease, and, consequently, choose the optimal treatment method. Recall that AIT patients have a higher risk of developing "thyroid cancer" than those who do not suffer from this disease [2,7,8,11]. Such development of the pathological process is based on genetic mechanisms. During histological and immunohistochemical examinations, it was noted that an increase in the expression of Ki-67, p53 and an increase in the expression of thyroglobulin in autoimmune thyroiditis are reflections of proliferation processes of thyrocytes. As you know, proliferation is a direct path to cellular dedifferentiation and dysplasia of thyrocytes. It is important to emphasize that with different forms of AIT there are changes of varying intensity [3,5,10,12].

The general principle of the treatment of autoimmune diseases is to suppress the activity of the immune system, to reduce the lymphoplasmacytic infiltration of the thyroid tissue. Functionally active mononuclear cells infiltrating the thyroid tissue also actively accumulate photosensitizers, just like tumor cells and microflora [4,7,13]. Consequently, it is possible to induce their apoptosis with the formation of apoptotic bodies, which macrophages recognize and phagocytose without damaging

neighboring healthy cells. The release of regulatory cytokines, together with the anti-inflammatory effect, makes it possible to test photodynamic therapy in the treatment of autoimmune thyroiditis [8,12,14].

In recent years, based on the revealed polyvalent effect of photodynamic therapy (PDT), laser PDT has been successfully applied in the treatment of benign and malignant skin neoplasms of various localizations, psoriasis, and inflammatory diseases [6,8,9,11]. The mechanism of action of photodynamic therapy is the selective accumulation of a light-sensitive substance in inflammatory tissue and pathogenic microorganisms and its activation by laser irradiation to produce active oxygen species that cause bactericidal effects without damaging healthy tissues. Photodynamic therapy has anti-inflammatory, antihistamine, desensitizing, and immunomodulatory effects [10,11,13]. The accumulated experience gives grounds to apply this method in the treatment of AIT. The photodynamic effect is local in nature, and the bactericidal effect is limited to the zone of laser irradiation, which avoids several side effects observed during antibiotic therapy [7,13,14]. From our point of view, at the present, it is quite justified to study the possibility of using the method of laser PDT in the treatment of patients with the diffuse form of autoimmune thyroiditis.

The purpose of the study

To study the nature and dynamics of changes in humoral and local immunity during laser photodynamic therapy in patients with diffuse forms of autoimmune thyroiditis.

Material and methods

The work is based on the results of laboratory examinations for the indices of humoral and local immunity of 160 patients hospitalized at the clinical base of the Scientific Center of Surgery named after Academician M.A.Topchubashov. Patients aged 16 to 74 years were admitted to the hospital in a planned manner at various times from the onset of the disease. Patients were divided into three age groups (I group-low age group (ages 16-35), II group-middle age group (ages 35-59), and III group-old patients (ages 60-74)).

To study the features of humoral and local immunity in 160 patients of different age groups with long-term autoimmune thyroiditis, a laboratory study of blood plasma was performed. In the samples information about immunoglobulins A, G, M (IgA, IgG, IgM), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) was determined. The dynamics of these laboratory parameters on the 7th and 15th days of treatment were studied in

patients of all three groups. These indicators were determined using reagents of the company "Vector-Best" (Russia). These reagents were obtained from the Scientific Research Immunology Laboratory. The material was taken before treatment, after 7 and 15 days from the start of treatment.

The functional activity of mononuclear cells of whole blood in an in vitro reaction was studied according to the daily spontaneous and mitogen-stimulated production of cytokines TNF- α and lactoferrin in the blood serum of AIT patients before the prescribed treatment. The possibility of using this approach to optimize the diagnosis and predict the state of cellular immunity with autoimmune thyroiditis was assessed. The concentration of α 2-MG was determined by quantitatively low-voltage immunoelectrophoresis in agarose gel plates using monospecific polyclonal antibodies to proteins. The level of Lactoferrin (Lf) and also TNF- α , IL-6, IFN, and hormones TSH and free T4 were investigated by the method of enzyme-linked immunosorbent assay (ELISA) using the "Vector Best" test systems.

We have studied the parameters of inflammatory cytokines, inflammatory mediators, immunoglobulins, and endothelial growth factors in the blood of patients with various forms of autoimmune thyroiditis. Statistical processing of the obtained data was carried out with the help of MS Excel-2016 software by the method of variational statistics. Statistical evaluation was performed using the Mann-Whitney U criterion with Bonferroni corrections.

Results and discussion

The level of TNF- α in blood plasma in all forms of thyroiditis was detected slightly higher in diffuse toxic goiter (DTG) and AIT in the decompensation stage, though it did not go beyond the reference values (5 μ g/l). IL-6 concentrations were significantly increased in DTG and AIT (18 or more times). Low levels of TNF- α in all groups of patients indicate long-term chronic inflammation.

Cytokines play an important role in modulating immune responses and initiating the immune process. Their role has been insufficiently studied and is often difficult to predict without taking into account other mediators - immune and hormonal. Regulatory cytokines can induce self-tolerance to thyroid antigens or, on the contrary, activate autoimmune processes in it. The study of the role of each specific cytokine in the context of a specific immune response in different functional states of the thyroid gland is of fundamental importance for the development of appropriate strategies for their modulation.

When examining patients with AIT, clinical signs of hormonal disorders were reflected by changes in the level of TSH and FT4. The con-

Tireoiditin forması	TTH mIU/ml	Ser. T ₄ pmol/L	TNF-α (pg/ml)	α ₂ -MG	IL-6	LF
control n = 40	1,37±0,16	16,38±0,71	0,9±0,2	1,89±0,6	0,5±0,1	1,06±0,07
I G. hypertrophic (n = 32)	15,7±2,15	8,4±1,50	2,5±0,3	2,81±0,19	11±1,1	1,49±0,10
II G. Atrophic (n = 28)	21,6±2,73	17,82±0,76	2,2±0,3	2,57±0,10	8,9±1,5	1,44±0,17
III G. Recurrence (n = 30)	14,14	11,2±2,7	1,1±0,1	2,37±0,10	9,4±0,9	1,88±0,15
IV G. DTG (n = 30)	0,03±0,01	59,08±6,18	1,9±0,2	2,85±0,10	7,7±0,9	1,71±0,12

* - the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 35-59;

- the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 60-74;

Statistical evaluation was performed using the Mann-Whitney U criterion with Bonferroni corrections.

centration of α₂-MG – 1,7±0,12 in the blood was increased in DTG and AIT by an average of 1,5 times. During treatment, its levels statistically significantly decreased in DTG and were somewhat lower than before treatment with AIT, although they remained significantly higher than in the control group (table 1).

The average TSH level in patients with AIT was 15±4,71 mIU/ml, the concentration of free thyroxine was T₃-10,26±0,66 and T₄-15,3±1,08 pmol/L, respectively.

In DTG and AIT, α₂-MG concentration in the blood serum before treatment was increased in the decompensation stage. During treatment, its levels statistically significantly decreased in DTG and were slightly lower than before treatment with AIT, although they remained significantly higher than in the control group. The LF content was significantly increased in AIT (1,3 times) and DTG (1,6 times).

The treatment did not significantly affect its concentration. The level of the proinflammatory cytokine TNF-α was slightly higher than normal in DTG and AIT in the decompensation stage, though it did not go beyond the reference values.

The cytotoxic effect in the thyroid gland is largely mediated by Th-1 lymphocytes, which ac-

tively produce TNF and especially IFN-γ. An excess of IFN-gamma induces the expression of MHC-P, the synthesis of chemokines and the expression of adhesion molecules, promotes the expansion of T- and B lymphocytes and macrophages into the thymus, supports the development of an inflammatory response, causes the progression of autoimmune processes and stimulates apoptosis of thymocytes. It is predicted that the hypersynthesis of proinflammatory cytokines in response to infectious agents is one of the trigger mechanisms of autoimmune pathological changes in the thyroid gland. Inducers of inflammation and autoimmune processes such as TNF-α and IL-6 make a somewhat smaller contribution to the pathogenesis of the disease. In this regard, the following fact is an indicator: we did not observe a complete normalization of the concentration of TNF-α and IL-6 in the blood of patients with DTG and AIT, despite the clinical effect. In our opinion, conservative treatment carried out for 4-6 months did not have a pronounced effect on the causes of the pathological process and only stopped their consequences.

One of the reasons for this situation may be dysregulation of the synthesis and functioning of polyfunctional immunoregulatory proteins. In particular, α₂-MG is an inhibitor of protein-

Table 1. Concentrations of immunoregulatory proteins and anti-inflammatory cytokines in the blood of patients with DTG and AIT

Form of thyroiditis	Indicators		
	TTH mIU/ml	T ₄ pmol/L	Anti TPO IU/mL
Hypertrophic (n = 32)	7,9 ± [3,33±5,29]	5,5 ± [3,2±4,5]	640 ± [27,7±30,38]
Atrophic (n = 28)	12,5 ± [3,35±6,2]	7,9 ± [3,0±4,1]	650 ± [28,13±33,43]
Recurrence (n = 30)	15,2 ± [3,73±7,8]	8,0 ± [2,1±3,18] *	690 ± [35,6±38,88] *

* - the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 35-59;

- the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 60-74;

Statistical evaluation was performed using the Mann-Whitney U criterion with Bonferroni corrections.

Table 2. Titers of serum Anti TPO in patients with different forms of autoimmune thyroiditis

Table 3.

Indicators of immunoglobulins in the blood of patients in different age groups

Age groups	Immunoglobulin indicators		
	IgA, g/l	IgM, g/l	IgG, g/l
I G. (n = 32) (ages 16-35)	4,31 [3,33-5,29]	3,85 [3,2-4,5]	29,04 [27,7-30,38]
II G. (n = 28) (ages 35-59)	4,78 [3,35-6,2]	3,55 [3,0-4,1]	30,78 [28,13-33,43]
III G. (n = 30) (ages 60-74)	6,27 [4,73-7,8]	2,64 [2,1-3,18]*	37,24 [35,6-38,88]*
DTG n=30	1,9±0,9	0,9±0,6	12,9±4,8

* - the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 35-59;

- the values of the quantitative feature in the mentioned group are statistically significant ($p < 0.05$) and differ from the values in the age group 60-74;

Statistical evaluation was performed using the Mann-Whitney U criterion with Bonferroni corrections.

ases released during inflammation, is involved in the recognition and presentation of infectious agents, regulation of proliferation and apoptosis, and tissue remodeling. Lf also has antibacterial and antiviral properties, regulates the synthesis of cytokines, and is a highly sensitive marker of inflammation. An excess of cytokines (especially IL-6 for $\alpha 2$ -MG and chemokine IL-8 for Lf) activates the synthesis of these proteins. At the same time, pronounced inflammation leads to the oxidation of $\alpha 2$ -MG molecules and their accumulation in blood circulation, which is confirmed by the results of our research.

The accumulation of $\alpha 2$ -MG in autoimmune diseases of the thyroid gland was as follows. As the patient's ages were increasing, we observed a tendency to an increase in the level of immunoglobulins. The IgA index in blood plasma was 4,31 [3,33-5,29] g/l in patients of the first age group (ages 16-35), 4,78 [3,35-6,2] g/l in patients of the middle age group (ages 35-59), and 6,27 [4,73-7,8] g/l in the elderly (ages 60-74), which is statistically significantly higher than in middle-aged patients ($p < 0,05$).

In the study of IgM, we did not note statistically significant differences among the studied groups, although there was a slight increase in the median values. IgM indices were 3,85 [3,2-4,5] g/l in the first age group, 3,55 [3,0-4,1] g/l in the second age group, and 2,64 [2,1-3,18] g/l in the group of elderly patients. When analyzing IgG indicators, there were statistically significantly high values ($p < 0,05$, for each of the comparison pairs with both groups) of these immunoglobulins in the elderly than in younger groups. IgG was equal to 29,04 [27,7-30,38] in patients of the first group, 30,78 [28,13-33,43] in the second group, and 37,24 [35,6-38,88] in the third group.

Thus, in elderly patients with long-term AIT, there is a tendency for TNF indices to decrease, which indicates the severity of the pathological

process. Increased immunoglobulin (IgA and IgG) levels are observed in elderly patients with a long-term inflammatory process in the thyroid gland. In this category of patients, changes in the immune system ($\alpha 2$ -MG -negative reactant of inflammation) negatively affect all physiological processes due to defective forms. This causes pathological changes controlled by a negative reactant of inflammation, including stimulation of cytokine synthesis. It is predicted that the higher the content of $\alpha 2$ -MG in AIT and DTG and the slower their normalization during treatment, the higher the probability of a relapse of the process. The situation is similar with Lf: the direction of its effect on the synthesis of cytokines is regulated by the balance of free and iron-bound forms of Lf. When T3 and T4 deficiency are observed during autoimmune changes in the thyroid gland, high levels of LF in the blood do not improve the situation, but rather support the inflammatory process and autoimmune changes. Accordingly, a decrease in LF concentration in the blood serum during treatment, especially a high number of cytokines, indicates a negative prognosis and a high risk of recurrence in the near future. In general, $\alpha 2$ -MG in DTG and AIT behaves like a positive late reactant of inflammation, which is slowly removed from the blood circulation; therefore the absence of normalization of this indicator in the blood after treatment is not informative.

Conclusion

The combined use of modern laser technology in the treatment of patients with autoimmune thyroiditis expands the possibilities of conservative therapy and completes the arsenal of effective methods of treatment of this disease. The simplicity of the methods, ease of application, reliability, the absence of thermal effects on the thyroid gland create ample opportunities for the application of this method in clinical practice.

References

1. Saprina T.V. General patterns and features of dysregulation of the immune system in endocrine diseases of autoimmune genesis: Thesis of MD, - 2014, - 388 p.
2. Kim K.W., Park Y.J., Kim E.H., et al. Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis // *Head Neck*. -2011.- Vol. 33(5), -P. 691-695
3. Saprina T.V., Prokhorenko T.S., Ryazantseva N.V. Immunological and morphological predictors of clinical heterogeneity in patients with Graves' disease (based on the results of a study of the operative material of the thyroid gland) // *Bulletin of Siberian Medicine*. - 2015. - T. 14, -№ 1, - P. 81-91. (in Russian)
4. Dzhikaev G. D. Morphological criteria for the diagnosis of chronic autoimmune thyroiditis. Thesis of MD. 117 p. Volgograd 2016.
5. Poloz T.L., Shevchenko S.P. Problems of cytological diagnosis of thyroid follicular tumors // *Siberian Journal of Oncology*. - 2011. - № 6 (48) - P.62-65. (in Russian)
6. Polyakov A.V., Kirillov Y.B., Aristarkhov V.G., Biryukov S.V. Indications and volume of surgical treatment for chronic autoimmune thyroiditis // *Actual problems of surgery and healthcare organization // Ryazan -1977* -p. 174-177 (in Russian)
7. Fomin D.K., Tararukhin O.B. Possibilities of two-indicator scintigraphy in the differential diagnosis of malignant and benign thyroid diseases // *Medical Radiology and Radiation Safety*. - 2010. - T.55, № 2. - P.39-42. (in Russian)
8. Fridman M.V., Demidchik Y.E., Meleshko O.I. Problems of interaction between doctors of different specialties at the stages of diagnosis of tumors and tumor-like conditions of the thyroid gland // *Medical panorama*. - 2004. - № 5. - P.19-21. (in Russian)
9. Fadeev V.V. Diseases of the thyroid gland in the regions of mild iodine deficiency // - M: Vidar, 2005. - 240 p.
10. Kharnas S.S., Mamaeva S.K. Long-term results and quality of life after surgical treatment of diffuse toxic goiter // *Endocrine Surgery*. - 2008. - № 1 (2). - P.10-14. (in Russian)
11. Charles R.P., Iezza G., Amendola E. et al. Mutationally activated BRAF (V600E) elicits papillary thyroid cancer in the adult mouse // *Cancer Res*. - 2011. - Vol.71. (11). - P.3863-3871.
12. Chen R.H., Chen W.C., Wang T.Y. Lack of association between pro-inflammatory cytokine (IL-6, IL-8 and TNF-alpha) gene polymorphisms and Graves' disease // *Int.J. Immunogenet*. 2005. 32(6).343-347
13. Stranadko E.Ph. Photodynamic therapy in ENT cancer. Moscow, 2016, p. 86 (in Russian)
14. DeMicco C, Ruf J., Chrestian M.A. et al. Immunohistochemical study of thyroid peroxidase in normal, hyperplastic, and neoplastic human thyroid tissues // *Cancer*. 1991. -Vol. 67, № 12. - P.3036-3041.