

ANNOTATION

of PhD thesis by **Akhmetova Venera Tursynovna**
on the topic "**Improving the study of heterogeneity of epitopes of
antithyroperoxidase autoantibodies in patients with autoimmune
diseases of the thyroid gland**"

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Relevance of the work: Autoimmune diseases of the thyroid gland are the most common organ-specific autoimmune diseases. In particular, approximately 1% of the population will develop diffuse toxic goiter during their lifetime, and ~15% of adult women will be diagnosed with autoimmune thyroiditis (Caturegli P. et. al., 2007, Hollowell J.G.et.al.,2002). During these pathologies autoimmune intersections occur, which are characterized by a combination of clinical, biochemical and serological manifestations typical for several diseases of this organ. This complex of symptoms is called the intersection syndrome or "overlap-syndrome" (Gendeleka G.F. et.al., 2014, Anaya J.M. et. al., 2016, Edit B. et. al., 2006)

The term overlap-syndrome means that one patient has the signs of two different autoimmune diseases that probably have a common pathogenesis. The factors that determine the balance between diffuse toxic goiter and autoimmune thyroiditis are unknown.

The main thyroid antigens that are important in the development of autoimmune diseases are thyroglobulin (TG), antibodies to thyroperoxidase (ATkTPO) and antibodies to the thyroid-stimulating hormone receptor (AT to RTG).

Antibodies to TPO and antibodies to the TSH receptor in the blood serum of patients with autoimmune thyroid diseases are directed to various sites of the enzyme. Epitope specificity to certain parts of the thyroid peroxidase molecule was studied by using monoclonal antibodies to thyroid peroxidase.

Identifying the causes of such heterogeneity of epitopes in patients with various thyroid disorders can clarify the pathophysiological mechanisms of the development of autoimmune thyroid diseases.

To this date it is known that linear and conformational epitopes are crucial for the development of autoimmune diseases of the thyroid gland. Available foreign studies persuasively testify that epitopes are not the same in different individuals, and at the same time the abundance of autoimmune thyroid diseases continues to increase, which requires further study.

The confirmation of the importance of studying specific epitopes has clinical significance because it can offer therapeutic possibilities through the use of monoclonal antibodies to thyroid peroxidase (Zubkov A.V. et. al., 2011 McLachlan S.M. et. al., 2017).

In recent years, there has been a noticeable progress in the study of the mechanism underlying autoimmune thyroid diseases. There have been some studies on the identification of T- and B-cell epitopes, in silico, in vitro, in vivo and in clinical experiments (Inaba H. et. al., 2006, Lee H.J. et. al., 2015).

Currently, the study of heterogeneity of epitopes of antithyroid peroxidase autoantibodies in patients with autoimmune diseases of the thyroid gland has not been carried out in conditions of unfavorable ecology and iodine deficiency. Based on the conducted research and meta-analysis, the key ideas of the pathogenesis and treatment of diffuse toxic goiter and autoimmune thyroiditis were proposed. Further research is needed to identify and clarify the shifts in the links of immunological systems, as well as a differential diagnosis between diffuse toxic goiter, autoimmune thyroiditis and overlap-syndrome, and based on that an algorithm can be developed for the diagnosis and treatment of autoimmune thyroid diseases, taking into account the heterogeneity of epitopes.

Cytokines also play an important role in the development and progression of autoimmune thyroid diseases. In order to understand the role of cytokines in the initiation or suppression of autoimmune thyroid diseases, it is important to understand the immunological events that trigger an autoimmune response, which ultimately leads to associated pathology. Cytokines modulate inflammation reactions of the immune system, regulating the growth, mobility and differentiation of lymphoid and non-lymphoid cells. IL-10 (IL-10) and tumor necrosis factor alpha (TNF- α) are of particular interest for studying the etiology of autoimmune thyroid diseases. (Balaji B.G. et. al., 2011)

The developed diagnostic algorithm based on the assessment of the prognostic significance of the heterogeneity of monoclonal antibody epitopes in various autoimmune thyroid diseases has significantly optimized diagnostic and therapeutic interventions in patients with autoimmune thyroid diseases.

Purpose of the research: To develop effective criteria for the diagnosis and treatment of autoimmune diseases of the thyroid gland by studying the heterogeneity of epitopes of antithyroid peroxidase autoantibodies in patients with AIHL using monoclonal antibodies to thyroid peroxidase.

Research objectives:

1. To conduct an epidemiological assessment of autoimmune diseases of the thyroid gland in the East, North, South and West Kazakhstan regions.
2. To evaluate the heterogeneity of epitopes of antithyroid peroxidase autoantibodies in patients with autoimmune thyroid diseases using monoclonal antibodies to thyroid peroxidase.
3. To establish the role of cytokines IL-10 and TNF- α in the formation of autoimmune diseases of the thyroid gland.
4. To develop an algorithm for the diagnosis and treatment of autoimmune thyroid diseases using monoclonal antibodies.

Research design:

A cross-sectional study included 398 patients with such diagnoses as diffuse toxic goiter, autoimmune thyroiditis and overlap-syndrome. Patients were included in the study in the period from January 1, 2016 to December 31, 2019.

The study participants were included from the list of patients observed by endocrinologists on an outpatient basis. Before deciding to include in the research each patient was consulted by two experts in the field of endocrinology. This consultation included verification of the diagnosis, objective clinical examination, thyroid function tests and ultrasound examination of the thyroid gland, if necessary, a fine needle biopsy of the thyroid gland was performed, followed by cytological examination by Papanicolaou to exclude malignant neoplasms. The same researchers examined each patient every six months to monitor his/her condition.

The functional state of the thyroid gland was studied: Thyroid-stimulating hormone (TSH), free thyroxine (svT4), antibodies to thyroperoxidase (AT to TPO), antibodies to the thyroid-stimulating hormone receptor (ATRTRG), and ultrasound examination (ultrasound) were performed for all patients before inclusion in the study and at each subsequent visit during the study period.

Epitopes in autoimmune diseases of the thyroid gland and the binding constants of thyroperoxidase with monoclonal antibodies were studied in the laboratory of immunological diagnostics of endocrine diseases "I. I. Mechnikov Research Institute of Vaccines and Serums", Moscow, the Russian Federation. We used 8 McAT for TPO, out of which five reacted with native TPO and the rest reacted with denatured TPO.

Inclusion criteria:

- The patients who are registered with an endocrinologist with autoimmune thyroid diseases in polyclinics;
- Elevated levels of antibodies to thyroperoxidase from 200 IU/ ml and higher in AIT;
- The availability of informed consent to participate in the study.

Exclusion criteria:

- Inflammatory diseases of the upper respiratory tract and other inflammatory diseases.
- Malignant neoplasms of the thyroid gland.
- The lack of informed consent to participate in the study or refusal to participate at any stage before the analysis is completed;

The study included 398 patients, the vast majority of patients were women in both the AIT subgroup (93.3%) and the DTZ subgroup (70.7%). The average age of patients was very similar: 47.77 years for patients with AIT and 45.75 years for patients with DTZ.

All statistical tests were carried out in the statistical software SPSS 20. Quantitative variables were presented as a median with the 25th- 75th percentile in cases of asymmetric distribution and as an average \pm standard deviation when

the data distribution was close to normal. Qualitative data were presented in the form of absolute figures and percentages. The Mann-Whitney U-test was used to compare quantitative variables, while the H²pirson criterion was used to compare qualitative variables. The significance level of the observed differences was presented as $p < 0.05$.

Materials and methods of research:

1. For the study the samples of blood sera were collected from the subjects with a laboratory and instrumentally confirmed diagnosis of autoimmune thyroid diseases.
2. The study of epitopes in autoimmune diseases of the thyroid gland and the binding constants of thyroid peroxidase with monoclonal antibodies and autoantibodies were carried out in the laboratory of immune endocrinology of the I.I.Mechnikov Research Institute, Moscow.
3. The determination of interleukins IL-10, TNF- α using solid-phase ELISA;
4. The ultrasound research of the thyroid gland and a fine needle biopsy of the thyroid gland with subsequent cytological examination by Papanicolau;
5. The hormonal study: thyroid-stimulating hormone, free T4, AT to thyroid peroxidase, antibodies to TSH receptors.

Scientific novelty:

1. The heterogeneity of epitopes recognized by antibodies to TPO was studied for the first time in patients with autoimmune diseases of the thyroid gland in Kazakhstan, in world thyroidology with overlap syndrome, we could not find a single study of the heterogeneity of ATkTPO epitopes. This study can be considered as the first attempt to get an idea of the specificity of epitopes in overlap syndrome.
2. It has been proven that pronounced inhibition of the binding of MCAT 82 in overlap syndrome can be considered as one of the potential markers of a favorable course of diffuse toxic goiter.
3. It has been established that elevated TNF- α indicators can be considered as predictors of the severity of the clinical course of autoimmune thyroid diseases. Statistically significant differences in the level of IL-10 between patients with hypo- and hyperthyroidism, functional status of the thyroid gland were not found statistically significant and correlative relationship.
4. An algorithm has been developed for diagnosing and treatment autoimmune thyroid diseases using monoclonal antibodies.

The main provisions of the dissertation research submitted for defense:

1. The indicators of the frequency of autoimmune thyroid diseases in the East, North, South and West Kazakhstan regions tend to increase.
2. The pronounced inhibition of the binding of MCAT 82 in the case of overlap-syndrome can be considered as one of the potential markers of a favorable

course of diffuse toxic goiter. Inhibition of the binding of McAT 63 in the serum of patients with autoimmune thyroiditis is more significant than those in the serum of patients with diffuse toxic goiter.

3. It has been established that elevated indicators can be considered as predictors of the severity of the clinical course of autoimmune thyroid diseases. Statistically significant differences in the level of IL-10 between patients with hypo- and hyperthyroidism, functional status of the thyroid gland were not found statistically significant and correlative relationship.

4. An algorithm has been developed for diagnosing and treatment autoimmune thyroid diseases using monoclonal antibodies.

Practical significance:

1. Diagnosis of the overlap syndrome using the diagnostic biomarker MkAT 82.

2. An algorithm is recommended for diagnosing and treatment autoimmune thyroid diseases using monoclonal antibodies.

Personal contribution of the author

The author independently analyzed the scientific literature on the topic of the dissertation, as well as statistical data from regional clinics.

The dissertation examined the specificity of autoantibodies to TPO by the method of competitive ELISA, summarized the results, and carried out statistical processing of the data obtained. All sections of the dissertation goals, objectives and research programs, collection and processing of material, development of the main provisions of the dissertation, findings, conclusions and practical recommendations are formulated and written by the author independently.

Conclusions:

1. The analysis of incidence rate for the period of the years 2016-2020 according to the performed screening revealed a total increase in the autoimmune thyroid diseases in East Kazakhstan for 46.8%, South Kazakhstan for 44.1%, North Kazakhstan for 45.5%, and West Kazakhstan for 43.61%. Autoimmune thyroid diseases are more common among people with an average age of 47.18 ± 14.99 years. The vast majority of patients were women in both the AIT subgroup (93.3%) and the DTZ subgroup (70.7%).

2. Pronounced inhibition of the binding of McAT 82 in overlap syndrome can be considered as one of the potential markers of a favorable course of diffuse toxic goiter. Autoantibodies in the blood serum of patients with autoimmune thyroiditis inhibited the binding of McAT 63 more significantly than those in the blood serum of patients with diffuse toxic goiter. This difference suggests that McAT 63 is a promising additional biomarker of AIT.

3. Statistical analysis showed that IL-10 is higher than normal in 19.4% of patients, 80.5% of patients have normal indicators. Statistically, IL-10 indicators are higher in patients diagnosed with autoimmune thyroiditis. No statistically

significant and correlative relationship was found between patients with hypo- and hyperthyroidism, functional status of the thyroid gland and place of residence after using the Pearson's criterion of consent χ^2 of statically significant differences in the level of TNF α between patients with hypo- and hyperthyroidism, functional status of the thyroid gland and place of residence. But at the same time, in the general group, TNF- α is elevated in 48.9% of patients, in 50.3% of patients, the indicators are normal.

4. An algorithm has been developed for diagnosing and treatment autoimmune thyroid diseases using monoclonal antibodies. The proposed algorithm allows for timely diagnosis of overlap syndrome based on pathogenetic differences, where it is recommended that if pronounced inhibition of MkAT82 is detected in diffuse toxic goiter, aggressive therapy with the development of such possible complications as hypoparathyroidism, tetany, severe hypothyroidism, paresis of the vocal cords is not required. The detection of pronounced inhibition of MkAT82 may have useful diagnostic value as an additional diagnostic tool for the diagnosis of overlap-syndrome at the earliest possible time, which may reduce the incidence of complications of autoimmune thyroid diseases.

Practical recommendations:

1. The study of the heterogeneity of epitopes of antithyroid peroxidase autoantibodies in patients with autoimmune diseases of the thyroid gland is recommended for use in practical medicine for the diagnosis of overlap syndrome using the diagnostic biomarker MkAT 82.
2. An algorithm is proposed for diagnosing and treatment autoimmune thyroid diseases using monoclonal antibodies.

Information about publications:

10 scientific papers have been published on the topic of the dissertation research, including:

- 1 publication in Scopus database (Journal of Clinical & Translational Endocrinology, Volume 27, March 2022, 100293) published by Elsevier Inc.;
- 3 publications recommended by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan (2 articles in the journal "Astana medical journals" and 1 article in the journal "Bulletin of KazNMU");
- 6 theses were published in the collection of a foreign conference.

Approbation of the dissertation:

The study materials were presented and discussed at the following conferences: "Proceedings of the 6th International Scientific and Practical Conference INTERNATIONAL FORUM: PROBLEMS AND SCIENTIFIC SOLUTIONS" Melbourne, Australia 2020;

"Proceedings of the 2nd International Scientific and Practical Conference GLOBAL AND REGIONAL ASPECTS OF SUSTAINABLE DEVELOPMENT" Copenhagen, Denmark 26-28.02.2021;

12-th International Congress on Autoimmunity, Athens, Greece, 28 May -1 June, 2021, A study on the immune system traits in the pathogenesis of Graves disease and autoimmune thyroiditis, № 1842;

13-th International Congress on Autoimmunity, Athens, Greece, 10-13 June, 2022 , Heterogeneity of antibody-reactive epitopes to thyroid peroxidase in patients with autoimmune diseases and overlap syndrome #372 Topic: AS45 #372 Topic: AS45, Thyroid autoimmunity;

VIII All-Russian Scientific and Practical Conference of Young Scientists and Students with International Participation "VolgaMedScience", the Federal State Budgetary Educational Institution "PIMU" of the Ministry of Health of Russia, March 17-18, 2022 in Nizhny Novgorod;

International Conference in Tokyo, Japan. Scientific advances and innovative approaches Proceedings of the I International Scientific and Practical Conference 01-02 December 2022;

Participation with the publication of the thesis of the XIII International Scientific and Practical Conference "Science and Education in the modern world: Challenges of the XXI century" Republic of Kazakhstan, Astana. 27-31 May 2023;

Participation with the publication of the thesis at 3 conferences of the republican level of international significance:

The Republican scientific and practical conference of Semey with international participation "Compulsory social health insurance - improving the efficiency of the healthcare system" on September 25, 2020;

The republican scientific and practical conference with international participation "Endocrinological diseases: modern approaches in diagnosis and treatment" of Semey, Republic of Kazakhstan November 14, 2019;

The conference of young scientists "Science and Health" dedicated to the 70th anniversary of corresponding member of the National Academy of Sciences of the Republic of Kazakhstan, Professor A.Z. Dyusupov and Associate Professor B.B. Dyusupova.

The scope and structure of the dissertation

The dissertation is presented on 88 pages, consists of an introduction, 3 sections of authors own research, findings, conclusions and practical recommendations, a bibliographic list including 219 titles of national, russian language and foreign literature, contains 7 tables, 13 figures.