DETERMINATION OF THE LEVELS AND POSSIBLE ASSOCIATIONS OF ALPHA2-MACROGLOBULIN WITH AUTOANTIBODIES IN THE SERUM OF PATIENTS WITH VARIOUS FORMS OF AUTOIMMUNE THYROIDITIS

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Antibodies to thyroid peroxidase (AB-TPO), antibodies to thyroglobulin (AB-TG), and the content of α2-macroglobulin (α2-MG) have been studied in serum samples of patients with autoimmune thyroiditis (AIT). All the patients were divided into 3 groups depending on age: 25–35, 36–50, 51–65 years. We found a significant change in the thyroid panel parameters in AIT, but without significant changes in the average concentration of α2-MG in the age groups of patients. This may be due to the accumulation and retention of complexes of defective forms of α2-MG in the circulation associated with their decreased ability to bind to receptors.

Key words: autoimmune thyroiditis; autoantibodies; thyroid peroxidase; thyroglobulin; α2-macroglobulin

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INTRODUCTION

Autoimmune thyroiditis (AIT) belongs to the group of complex polygenic diseases; its development is determined by many endogenous (including genetic) and exogenous factors leading to autoimmune damage to the thyroid gland. Hashimoto's thyroiditis (HT) is a common form of AIT [1–3]. HT is caused by autoimmune destruction of the thyroid gland, where CD8+ T cells cause the death of thyroid follicular cells. The release of interferon-gamma leads to the attraction and activation of macrophages [4]. Inflammation progression is accompanied by thyroid follicular damage and disruption. Thyroid follicular disruption may result in appearance of hyperthyroidism symptoms. However, the disease progression accompanied by fibrosis of the thyroid gland leads to a decrease in its size with the subsequent development of symptoms of hypothyroidism [5]. Thyroid hormones affect virtually every organ system in the body, including the heart, central nervous system, autonomic nervous system, bones, gastrointestinal tract, and metabolism. Thyroid hormones bind to their nuclear receptor and activate gene expression leading to the increase in the rate of metabolism and thermogenesis [6]. Although the key links in the pathogenesis of AIT are still not fully understood, a well-known feature of this organ-specific pathology is the presence of antibodies to thyroid tissue. Autoantibodies to thyroglobulin (AB-TG), colloid, and thyroid peroxidase (microsomes) of the thyroid gland (AB-TPO) represent a pathogenetic factor of the disease. AB-TG and AB-TPO belong to the class of immunoglobulin G (IgG). Both have high affinity for the corresponding antigens. Unlike AB-TG AB-TPO can activate complement and are capable of causing thyroid cell damage due to antibody-dependent cell-mediated cytotoxicity. T cell-mediated cytotoxicity and activation of apoptotic pathways are known to influence disease outcome [7]. Autoantibodies, interacting with follicular cells, cause their cytolysis; binding to thyroglobulin, peroxidase, preventing the absorption of iodine by these proteins, impairs iodine metabolism in the thyroid gland, thus leading to impaired production of thyroid hormones [8]. HT is accompanied by infiltration of the thyroid gland with subsequent induction of the inflammatory process through the production of proinflammatory cytokines. Some of these cytokines are capable of causing cytolysis of glandular cells; this ultimately leads to the development of pathology and clinical manifestations [9, 10]. The observed imbalance in cytokine synthesis is a consequence of dysfunction of immunoregulatory proteins. These proteins include the potentially immunogenic alpha-2-macroglobulin (α2-MG) [11]. It has been found that the concentration of the α2-MG complex with IgG increases during inflammatory processes and autoimmune diseases. In this case, severe inflammation leads to the oxidation of α2-MG molecules and their accumulation in the circulation. Such defective forms have altered affinity for receptors and transported cytokines [12].

It should also be noted that in addition to the transport of cytokines, growth factors, zinc, and inhibition of the activity of some metalloproteinases, α2-MG can stimulate transcription of various genes necessary for cell proliferation or hypertrophy [13]. Various stimuli can induce structural changes of the molecular forms of α2-MG. Thus, the precise molecular forms of α2-MG circulating in biological fluids and associated with health and disease remain to be elucidated [14]. Certain evidence

exists in the literature that post-translational modification of α2-MG makes the protein highly immunogenic; this can aggravate the autoimmune process by reducing the ability of the protein to bind and utilize regulatory substances [15]. α2-MG is an estrogen-dependent protein; in women of reproductive age its average level is higher than in men. This may be due to the estrogen effect increasing IFN-γ levels and IFN-γ-mediated inflammatory effects, including the production of inflammatory mediators (cyclooxygenase (Cox2) activation) [16, 17]. All of these α2-MG functions confirm the important role of α2-MG in the modulation of immune and inflammatory reactions. In this regard, it seemed interesting to study the level of α2-MG in AIT, which was the goal of this study.

MATERIALS AND METHODS

A single-center, open, prospective, non-randomized study was conducted using 170 patients with autoimmune thyroiditis (64 men and 106 women aged 25 to 65 years). Inclusion criteria were: patients with a primary diagnosis of AIT without concomitant allergic or other autoimmune diseases. Exclusion criteria were: chronic inflammatory processes affecting the immunological status of the patient, as well as pregnancy and lactation, and diagnosed comorbid pathology. The comparison group consisted of 65 people aged from 20 to 65 years (26 men and 39 women) without thyroid pathologies and other autoimmune diseases. Using results of analysis of clinical and laboratory studies with determination of the content of thyroid-stimulating hormone (TSH), free triiodothyronine (T3) and free thyroxine (T4) patients into divided into 2 groups:

group 1 — 74 patients with the manifest form of the disease (MAIT);

group 2 — 96 patients with the subclinical form of the disease (SAIT).

The diagnosis of AIT was confirmed by generally accepted clinical and laboratory-instrumental methods. The clinical picture of MAIT consisted of the following patient complaints: myxedematous edema — circles under the eyes, obesity, difficulty breathing, change in voice, drowsiness, slow thinking, emotionality, tendency to constipation or diarrhea, loss of sensation in the limbs, thinning and hair loss, disruption or cessation of menstruation. In this group of patients, an increase of TSH levels and a decrease in the levels of the thyroid hormones T3 and T4, as well as an increase in AT-TG and AT-TPO titers were observed.

SAIT was diagnosed on the basis of an increase in the level of TSH while the T3 and T4 levels remained within normal limits. The clinical picture for this form is characterized by "erased manifestations". In all participants, the level of α2-MG was determined using the immunoturbometric method. It is based on the reaction between α2-MG and polyclonal antiserum in the presence of polyethylene glycol. Reagents from Sentinel (Italy) were used for determination. Organ-specific antibodies, thyroid hormones — T3, T4 and TSH were determined by the immunochemiluminescence method using an IMMULITE 2000 Xpi device (Siemens Healthcare Diagnostics Inc., USA). Changes in α2-MG content and indicators of the functional state of the thyroid gland were compared by dividing patients into 3 age groups (25–35, 36–50, 51–65 years). The age-group subdivision has been made on the basis of literature data, which mainly describe these age groups and according to the literature the disease manifests itself and/or worsens either in the post-pubertal period, or in middle or adulthood [18–21].

Statistical analysis was performed using the Statsoft STATISTICA 12 software package. Data are presented as medians, upper and lower quartiles. Intergroup comparisons on quantitative indicators were carried out using the non-parametric Mann-Whitney rank test, taking into account the difference in the distribution of the analyzed indicators from normal. Differences were considered statistically significant at $p<0.05$.

RESULTS

According to the values of thyroid status parameters AIT patients were subdivided into two groups: patients with subclinical AIT (SAIT) and manifested AIT (MAIT) (Table 1).

Determination of organ-specific autoantibodies showed that in MAIT patients AB-TG and AB-TPO were statistically significantly elevated; the median values were, respectively, 470.0 (381; 527) U/ml and

Table 1. Serum thyroid hormone and TSH concentrations in patients with autoimmune thyroiditis, Me (Q_{25}; Q_{75})

Parameters	Comparison group (n=65)	AIT (n=170)	Subclinical form (SAIT) (n=96)	Manifested form (MAIT) (n=74)
T3 free, pg/ml	2.4 (2.1; 2.6)	1.5* (1.2; 2.5)	2.4 (1.9; 2.7)	1.2*# (1.1; 1.2)
T4 free, ng/dl	1.9 (1.5; 2.3)	1.6* (0.8; 2.0)	1.9 (1.8; 2.1)	0.8*# (0.7; 0.8)
TSH, U/l	2.1 (1.3; 2.3)	7.5* (5.6; 18.5)	4.2* (3.5; 4.6)	19.0*# (16.1; 24.3)

* – Statistically significant differences ($p<0.05$) as compared to the comparison group; # – statistically significant differences ($p<0.05$) as compared to the group of SAIT patients.

530.5 (458; 566) U/ml as compared to similar parameters in the control group, 16(13; 30) U/ml (p<0.001) and 20(13; 25) U/ml (p<0.001), respectively.

In SAIT patients, the levels of AB-TG and AB-TPO were, respectively, 456 (394.5; 543.5) U/ml and 523(464; 568) U/ml and were also statistically significantly higher than those in the control group — 16(13; 30) U/ml and 20(13; 25) U/ml (p<0.001). There were no statistically significant intergroup differences in this indicator in patients with different clinical forms of hypothyroidism (MAIT and SAIT).

In order to identify any significant association with indicators of thyroid status, the content of α2-MG was determined in all studied groups. In the total group of AIT patients, the concentration of α2-MG was statistically significantly higher (p=0.008) as compared with its concentration in the control group: 2.6 (2.4; 2.9) g/l and 1.6 (1.4; 2.0) g/l, respectively. Comparison of the concentration of α2-MG in patients with various clinical forms of hypothyroidism indicated significantly higher values (p=0.031) in MAIT patients (2.8(2.5; 3.3) g/l) as compared with the corresponding indicator in the group of SAIT patients (2.5(2.3; 2.9) g/l). In both forms of the disease, the concentration of α2-MG was significantly higher than that in the control group (p<0.001 for both comparisons).

In order to find out the nature of changes in α2-MG depending on the studied indicators of the functional state of the thyroid gland, patients were subdivided into 3 age-groups (Table 2).

DISCUSSION

The results of the studied thyroid parameters in SAIT and MAIT patients of different age groups showed the most pronounced changes in SAIT patients of different age subgroups. In SAIT patients the high levels of AB-TPO were detected in the age group 25–35 years (Table 2). In MAIT patients, the difference in the level of thyroid parameters was significantly less. There was only a statistically significant decrease in TSH levels in MAIT patients of the older age group (51–65 years) relative to the subgroup of patients aged 25–35 years. In the case of AB-TG, the highest values were in MAIT patients of the younger age group. It has been noted above that, unlike AB-TG, AB-TPO can activate complement with subsequent damage to the thyroid gland. It can be assumed that high titers of AB-TPO in the initial stages of the disease, especially in the young subgroup, trigger antibody-dependent cell damage, which subsequently induces the formation of AB-TG. Under these changes in the subclinical course, the content of α2-MG in different age groups did not differ significantly; only in MAIT patients of the middle age group this parameter increased. It is interesting that in the presence of antibody-dependent cell damage, there was no significant difference in the average level of α2-MG in patients of different age groups and in various clinical forms; however, differences were identified in the absolute protein content in the studied groups of patients, and all they were elevated over control values. The data available in the literature are not entirely clear. For example, Maklakova et al. reported about an increased content of immunoregulatory α2-MG in AIT hypothyroidism; the content of α2-MG remained elevated even after achieving the euthyroid state, which could be regarded as a risk factor for relapse [22]. In another study by Maklakova et al. an increase in α2-MG levels has been demonstrated in newly diagnosed or recurrent Graves' disease [23].

Shokal et al. have also shown that increased values of α2-MG make a certain contribution to the process of inflammation in immune disorders. Lisowska-Myjak et al. obtained results indicating an increase in α2-MG under conditions of decreased T3 and T4 in the last trimester of pregnancy [25]. On the other hand, a proteomic analysis of blood plasma from patients with hypothyroidism performed by Alfadda et al. revealed a decrease in α2-MG in the hypothyroid state compared to the euthyroid

Table 2. Characteristics of biochemical parameters in serum of SAIT and MAIT patients subdivided into three age groups, Me (25%–75%)

Parameters	SAIT			MAIT		
	25–35	36–50	51–65	25–35	36–50	51–65
	n=29	n=27	n=40	n=10	n=23	n=41
T3 free, pg/ml	2.30 (1.90–2.60)	2.30 (2.00–2.50)	2.40 (1.85–3.05)	1.14 (1.12–1.15)	1.18 (1.10–1.19)	1.15 (1.13–1.18)
T4 free, ng/dl	1.90 (1.60–2.10)	1.90 (1.80–2.10)	1.90 (1.80–2.10)	0.75 (0.74–0.78)	0.76 (0.74–0.78)	0.76 (0.75–0.77)
TSH, U/l	6.50 (5.40–6.90)	5.40* (4.50–6.50)	5.80 (4.50–6.95)	21.85 (16.00–26.50)	18.60 (16.40–24.60)	19.00* (16.00–24.00)
AB-TG, U/ml	456.0 (394.0–524.0)	486.0 (415.0–611.0)	452.0 (390.5–538.0)	492.0 (425.0–522.0)	425.0 (356.0–560.0)	479.0 (432.0–523.0)
AB-TPO, U/ml	561.0 (515.0–604.0)	531.0 (468.0–578.0)	487.0* (457.0–562.0)	527.5 (453.0–604.0)	524.0 (452.0–569.0)	531.0 (463.0–564.0)
α2-MG, g/l	2.50 (2.40–2.80)	2.60 (2.30–2.90)	2.50 (2.35–2.90)	2.55 (2.40–2.70)	2.80 (2.60–3.30)	2.60 (2.60–3.10)

* – p<0.05–0.001 as compared with patients in the age group 25–35 years within each clinical form of AIT.

state [26]. According to our assumptions, accumulation and retention of complexes of the defective forms of α2-MG in the circulation due to a decrease in their ability to bind to receptors may account for the observed results. Most disease-related studies have focused on the ability of α2-MG to inactivate active proteases and its ability to bind cytokines, while other functional capacities of α2-MG still remain better understanding.

CONCLUSIONS

1. According to the results of the study, the serum level of α2-MG significantly increased both in the general group of patients compared to control values, and in the manifest form of the disease as compared to the subclinical one.

2. Dividing patients into three different age subgroups, we have not found a significant association between changes in the serum α2-MG concentrations and the level of thyroid panel parameters, which would allow us to consider α2-MG as a candidate biomarker for the worsening of the autoimmune process in AIT.

3. Elevated levels of α2-MG in all studied age groups of patients indicate the persistence of immune system dysfunction.

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COMPLIANCE WITH ETHICAL STANDARDS

This study was approved by the Ethics Committee of the Azerbaijan Medical University (extract No. 12/AMU/IEC/02/07/2020). All participants gave voluntary informed consent for the study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ОПРЕДЕЛЕНИЕ СОДЕРЖАНИЯ И ВОЗМОЖНЫХ АССОЦИАЦИЙ α2-МАКРОГЛОБУЛИНА С АУТОАНТИТЕЛАМИ В СЫВОРОТКЕ КРОВИ ПАЦИЕНТОВ С РАЗЛИЧНЫМИ ФОРМАМИ АУТОИММУННОГО ТИРЕОИДИТА

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В сыворотке крови пациентов с аутоиммунным тиреодитом (АИТ) исследовали антитела к тиреопероксидазе (АТ-ТПО), антитела к тиреоглобулину (АТ-ТГ), а также содержание α2-макроглобулина (α2-МГ). Все пациенты были распределены на 3 группы в зависимости от возраста: 25–35, 36–50, 51–65 лет. Мы обнаружили значимое изменение показателей тиреоидной панели при АИТ, но без значительных изменений средней концентрации α2-МГ в возрастных группах пациентов, что, предположительно, может быть результатом накопления и задержки комплексов дефектных форм α2-МГ в циркуляции за счёт снижения их способности связываться с рецепторами.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: аутоиммунный тиреоидит; аутоантитела; тиреопероксидаза; тиреоглобулин; α2-макроглобулин

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