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ABSTRACT

of the dissertation for the degree of Philosophy
in Medicine

STATE OF THE IMMUNE SYSTEM AND MICRONUTRIENTS EXCHANGE DURING AUTOIMMUNE THYROIDITIS IN CHILDREN

Speciality: 3220.01 – Pediatrics

Field of science: Medicine

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GENERAL DESCRİPTİON OF THE WORK

Relevance of the topic. Autoimmune thyroiditis (AIT), which is one of the most common diseases among thyroid gland (TG) diseases, is considered one of the urgent problems of health care because it has a significant impact on the somatic condition, reproductive health, intellectual level and quality of life of the population. Autoimmune thyroiditis is one of the main causes of hypothyroidism even in iodine-rich regions of the world[1].

According to the data of recent years, 5% of the world's population suffers from AIT, and the frequency of its occurrence among women is 4-10 times higher than that of men. The prevalence of AIT varies by ethnicity, environmental factors, age, and gender. At the same time, as the age increases among the population, an increase in morbidity is also observed[2].

The incidence of AIT in the pediatric population is 5-6%[3]. In 70% of cases, this disease is observed in the inheritance of children with AIT [4].

79% of the basis of this pathology includes genetic predisposition, and 21% environmental factors[5]. In genetically predisposed

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children with AIT, the main factor in the onset of the disease is the violation of immunological control mechanisms. The hereditary nature of the disease is confirmed by data on the high frequency of the disease in close relatives[6].

In the modern literature, it is noted that in the pathogenesis of AIT, there are changes in the body's microelements as well as the immune system [7].

Some microelements involved in many vital mechanisms in the body are necessary for the development, growth, physiology of the body, participation in many physiological processes in the thyroid gland tissue, both synthesis and metabolism of thyroid hormones [8]. Since these microelements are not synthesized in the body, they must be taken mainly through food and nutritional supplements on a regular basis.

The role of selenium, zinc, copper, iodine and other microelements in the hemostasis of thyroid hormones has been revealed by many scientists, but their role in thyroid pathologies is still being studied [9].

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Despite the great achievements in the pathogenesis, clinic and treatment of AIT, the study of the scientific literature on this topic shows that the numerous changes in the immune system and microelements metabolism during AIT in children are sometimes ambiguous and even contradictory. Thus, the use of preparations containing microelements (especially selenium-containing) preparations in improving the treatment of children with AIT creates the basis for an individual approach to the treatment modification of the disease.

The relevance of the above-mentioned problem reveals the necessity of continuing scientific research in this direction in the field of medicine. It is in this direction that it is possible to clarify the existing ideas about the causes and course of the disease, its pathogenesis, to develop recommendations for autoimmune thyroiditis in children, to establish the foundations for correcting and improving treatment and prevention measures.

Object and subject of research:

In the study, 83 patients diagnosed with autoimmune thyroiditis and 22 patients diagnosed with diffuse non-toxic goiter were the object of the study, the interaction of thyroid hormones, immune system indicators and microelements in these patients was studied as the subject of the study.

Purpose of the study:

Comparative assessment of the characteristics of thyroid status, immune system and microelements metabolism in children with autoimmune thyroiditis.

The tasks of the research:

1. To assess the dynamics of the clinical and functional status of children with autoimmune thyroiditis, the titer of thyroid hormones (sT3, sT4), thyroid stimulating hormone (TSH), Anti-TPO and Anti-TG antibodies.

2. To determine the titer of CD3+, CD4+, CD8+, CD 19+, CD4/CD8+ lymphocytes and IL-1β, IL-6, TNF-α cytokines in children with autoimmune thyroiditis.

3. To determine the level of microelements in blood serum (selenium, zinc, copper) and urine (iodine) of children with autoimmune thyroiditis.

4. To study the interaction of thyroid status, immune homeostasis and metabolism of microelements in children with autoimmune thyroiditis.

5. To evaluate the effectiveness of microelements in improving the treatment of autoimmune thyroiditis in children based on the results of the examinations carried out.

Research methods: Research methods include clinical, laboratory, instrumental, and statistical methods.

Provisions of the dissertation submitted for defense:

1. An evaluation of the clinical manifestations and diagnostic markers of AIT among children was conducted and it was found that they do not depend on the functional state of the thyroid gland.

2. The clinical-diagnostic significance of CD markers and interleukins in children with AIT has been determined.

3. In children with autoimmune thyroiditis, an imbalance of microelements has been identified, a comprehensive and objective assessment of which has diagnostic value.

4. It is considered appropriate to include selenium in the complex treatment of children with autoimmune thyroiditis, which made it possible to eliminate microelement imbalance, normalize the activity of the immune system, and increase the effectiveness of treatment.

Scientific novelty of the work:

– By evaluating the state of the immune system during autoimmune thyroiditis in children, the significant effect of high concentrations of IL-1β, TNF-α and especially IL-6 interleukins on the dynamics of the increase in the titer of thyroid peroxidase antibody was studied.

– A complex assessment of the level of microelements in children during autoimmune thyroiditis was carried out, and it was found that the level of selenium in the blood serum is low in the euthyroid functional state of the thyroid gland.

– Correlations of immune system indicators, microelements, thyroid hormones and antibodies were studied in children.

– The effectiveness of the selenium preparation in the treatment of autoimmune thyroiditis in children has been substantiated.

Practical significance: The importance of determining the level of the selenium in children with AIT has been substantiated. By evaluating the clinical and functional status of children with autoimmune thyroiditis, studying and evaluating the interaction between thyroid status, immune system indicators, and indicators of microelements metabolism, the possibility of prescribing selenium in the treatment of AIT in children has been substantiated. The obtained research materials provide a basis for making practical recommendations on the combined treatment and prevention of autoimmune thyroiditis in children.

Approbation of the research and its application in practice:

The main provisions, data and results of the dissertation were presented at the meetings of the Azerbaijan Society of Allergists, Immunologists and Immunorehabilitators, the Azerbaijan Society of Pediatric Endocrinologists, at the 1st International Scientific and Practical Conference "Azerbaijan and Turkey Universities: Education, Science, Technology" (December 18-20, 2019), at the conference Modern Aspects of Allergology and Clinical Immunology (November 23, 2019), at the "XIII Republic International Scientific Conference of Doctoral Students and Young Researchers" dedicated to the 650th anniversary of Imadaddin Nasimi (December 3, 2019), at the 1st Congress of Pediatric Endocrinologists (July 2, 2022). The results of the research are applied in the practical work of the Educational- Therapeutic Clinic of AMU and in the treatment-diagnostic and teaching process of the "II Children's diseases" department.

Published scientific works: According to the results of the research, 14 scientific works have been published, 8 of them are articles and 6 are theses.

The organization in which the dissertation work was carried out: Dissertation work was performed at the "II Children's Diseases" department of AMU, the Scientific Research Immunology

Laboratory of Azerbaijan Medical University, the Educational-Therapeutic Clinic of the Azerbaijan Medical University, the "Diagnosis Medical Center" LLC, the "Baku Clinic" LLC.

The total volume of the dissertation with a sign indicating the volume of the structural sections of the dissertation separately:

The dissertation work consists of 158 pages (211931 characters) written in Azerbaijani, including an introduction (7833 characters), a literature review (64572 characters), materials and methods (18233 characters), Chapter 3 (21046 characters), Chapter 4 (25758 characters), Chapter 5 (23248 characters), conclusion (48276 characters), results (2367 characters), practical recommendations (598 characters), and a list of literature sources and conditional abbreviations. The dissertation work consists of a literature list that includes 181 literature sources. Of these sources, 6 were cited by local authors, 3 by turkish, 2 by russian, and 169 by authors writing in english. The work is illustrated with 22 tables and 7 graphs.

MATERIALS AND METHODS OF THE RESEARCH

In accordance with the goals and objectives, the dissertation work was carried out at the Department of "Children's Diseases II" of AMU, the Educational Therapeutic Clinic of Azerbaijan Medical University, the Scientific-Research Immunology Laboratory of Azerbaijan Medical University, "Diagnostic Medical Center" LLC, and "Baku (Diamed) Clinic" LLC. 120 children aged 3-18 (11.8±0.3) were included in the study.

During the study, complex examination of patients, as well as general clinical, instrumental and laboratory diagnostic methods were carried out, determination of the level of microelements in blood (selenium, zinc, copper) and urine (iodine), membrane markers of immunocompetent cells in blood (CD3+, CD4+, CD8+, CD19+, CD4/CD8+), cytokines (IL-1b, IL-6, TNF-a) and ultrasound examination of the thyroid gland.

All children included in the study were selected according to the inclusion criteria.

The inclusion criteria are as follows:

- Other endocrine diseases except autoimmune thyroiditis and diffuse non-toxic goiter disease are denied;
- Allergic diseases in the anamnesis are denied;
- Children not receiving glucocorticoid treatment;
- Children who do not take nutritional supplements containing microelements that we have investigated.

Children with autoimmune thyroiditis included in the study were excluded based on the following criteria:

- Children who did not take thyroxine and microelements supplements as prescribed during the 6-month follow-up period.

Children included in the control group were selected based on the following criteria:

- Autoimmune thyroiditis and diffuse non-toxic goiter disease are denied;
- Allergic diseases in the anamnesis are denied;
- Other endocrine diseases in the anamnesis are denied.

Children were selected based on the criteria of the World Health Organization (WHO) and the recommendations of the American Thyroid Association[10], referring to the main diagnostic criteria of autoimmune thyroiditis (AIT) in children and adolescents.

During the research, if thyroid gland diseases are suspected in children, the anamnesis was carefully collected and information received from both the child himself and his parents was taken into account. Anthropometric examinations were carried out in children, indicators of physical development were studied. In addition, the clinical symptoms of thyroid gland diseases such as discomfort in the neck, sleep disturbance, hair loss, mood disorders, and memory impairment were studied. Anamnesis of the disease, effectiveness of previous treatment, etc. was analyzed.

[10] [Darendeliler, F. Temel çocuk endokrinolojisi ve diyabet/ F.Darendeliler, Z.Aycan, C.Kara, S.Özen, E.Eren. – İstanbul. – Vizyon Basımevi, – 2023. – 854]

Taking into account the polymorphism of the manifestations of functional disorders of the thyroid gland, mandatory palpation of the gland was carried out. In the palpation examination, the dimensions of the thyroid gland in children with AIT corresponded to the I and II degree of growth of its parts.

The obtained height indicators were evaluated by calculating the deviations from the average level, except for the percentile curves, using the centile table. The delay in height was determined by the lag of body length in comparison with the average indicators - from 1 SDS to 2 SDS (Standard Deviation Score*-shows how many standard (sigmal) deviations from the difference between the average mathematical and measured indicator), and short stature - more than 2 SDS. SDS = (height – average height) / SD, where average height is the average height for a certain gender and age, SD – indicates the standard deviation of height for this gender and age (found in the table). The diagnosis of tall stature was made based on the fact that the body length is above 2 or more SD compared to the average indicators.

Body mass indicators were assessed using the body mass index (BMI). BMI = mass (kg) / height (m2). BMI < 18.5 - low, 18.5-24.9 - normal, 25-29.9 - overweight, 30 and above - obesity.

Ultrasound examination is a highly informative, non-invasive and cost-effective examination method for evaluating the anatomy, exact dimensions and structure of the thyroid gland. The main indication for ultrasound is applied when differential diagnosis of changes in the structure of the thyroid gland, as well as when it is not possible to obtain reliable results during palpation examination of the thyroid gland dimensions. The USM was carried out using a Samsung device with a sectoral transmitter with a frequency of 7.5 MQh and a scanning surface length of 4.5-1.0 cm. The exostructure and exosensitivity of the thyroid gland were evaluated in order to visualize the structure of the thyroid tissue.

The determination of the concentration of thyroid hormones in the blood is considered important for giving a final opinion on the functional state of the thyroid gland. In the study, the concentration of free T3, free T4, TSH, Anti-TPO and Anti-TG antibodies was determined by Chemiluminescence Immunoassay (CLIA).

During the determination of immune indicators, the relative and absolute number of CD markers was carried out by the flow cytofluorimetry method on the "Coulter Epix XL" device of the Beckton Dickinson (USA) company. Cell Quest software was used to process the results of the examination.

The concentration of anti-inflammatory cytokines IL-1, IL-6 and TNF-α in blood serum of healthy children and children with autoimmune thyroiditis and diffuse non-toxic goiter was determined by ELISA method using BIOSOURCE kits in ChemWell device.

The amount of micronutrients selenium, zinc, copper in the blood serum and iodine in urine was determined of children in all three groups.

The concentration of selenium microelements was determined on a Varian AA240-Duo Zeeman atomic-absorption spectrometer. The concentration of microelements zinc in blood serum was determined by the photometric method on a Beckman Coulter AU-400 device. The concentration of copper microelement was determined by the colorimetric method in the StatFax device.

The marker of the amount of iodine in the body is considered to be the assessment of the amount of iodine excreted in the urine (ioduria). Determination of iodine in urine was carried out by selective photometric method.

In the course of the study, U-Wilcoxon-Manna-Whitney (U-Mann-Whitney), KU-Kruskal-Wallis (KU-Kruskal-Wallis) in independent groups, t-Wilcoxon (T-Wilcoxon) pair criteria were applied in dependent groups (before and after treatment) for comparison of quantitative indicators of the obtained series during statistical analysis. Pearson Chi-Square (Pearson Chi-Square) was used in the analysis of quality indicators. To study the influence of the investigated factors on the result, dispersion analysis was used (ANOVA test), in which the results were evaluated by the F-Fisher criterion. To detect the dependence between the indicators, a correlation analysis of ρ-Spirmen (ρ-Spearman) was carried out.

All calculations were rejected at the level of “0” $p < 0.05$, performed in the EXCEL-2016 spreadsheet and the SPSS-22 package program.

RESEARCH RESULTS AND THEIR DISCUSSION

120 children aged 3-18 (11.8±0.3) (36 boys and 84 girls) were involved in the study. Patients were divided into 3 groups, the main group included 83 children (22 boys-26.5% and 61 girls - 73.5%) with autoimmune thyroiditis, the comparison group included 22 children (8 boys -36.4% and 14 girls – 63.6%) with diffuse non-toxic goiter, and the control group included 15 practically healthy children (6 boys - 40% and 9 girls - 60%). No differences were found in the subgroup comparison according to gender (p=0.448).

In order to determine the frequency of AIT according to age groups, children were divided into the following age groups: pres-chool age (3-6 years - 5 people), primary school age (7-11 years - 28 people) and secondary school age (12 - 18 years old - 50 people). Among children with AIT, the group of secondary school students (60.3%) had a wide composition. In the diffuse non-toxic goiter group, 15 (68.2%) children were in secondary school. The results obtained during the comparative analysis by age groups in both groups of sick children were not statistically significant (p=0.405).

In the height examination of children with AIT, 59 children (71.1%) had height for age, 22 people (26.5%) had height development retardation, and 2 people (2.4%) had tall stature. Height for age was found in 16 children (72.72%) and height development retardation in 6 (27.27%) children with DNTG. Statistical calculations were not significant during the assessment of height development in both groups of sick children (p=0.833).

In the study, no statistically significant differences were found when comparing both groups of sick children during the assessment of body mass index (p=0.710).

As a result of statistical analysis of clinical symptoms in children in the AIT and DNTG groups using the Person criterion, it was clear that throat discomfort was more common in children diagnosed with DNTG (p_x^2=0.060). Complaints of sleep disturbance were more common in the DNTG group compared to the DNTG

group (p_x^2=0.001). Children in the DNTG group included in the study complained of hair loss more often than in the AIT group (p_x^2=0.021). Statistically correct results were also obtained during intergroup comparisons on the complaint of mood disturbance, and children with DNTG prevailed (p_x^2= <0.001). Finally, during the comparative analysis on the complaint of memory impairment, children suffering from this complaint were more common in the AIT group (p_x^2=0.004).

Many of the emerging pathological symptoms were discovered only during an active anamnestic survey of children and their parents. Based on the character and psychological characteristics of childhood, children of this age did not pay special attention to the subjective parts. This opinion is confirmed by the fact that in the majority of children who did not have specific complaints before, the general mental mood improved, physical activity increased, and memory and success indicators improved against the background of treatment with selenium.

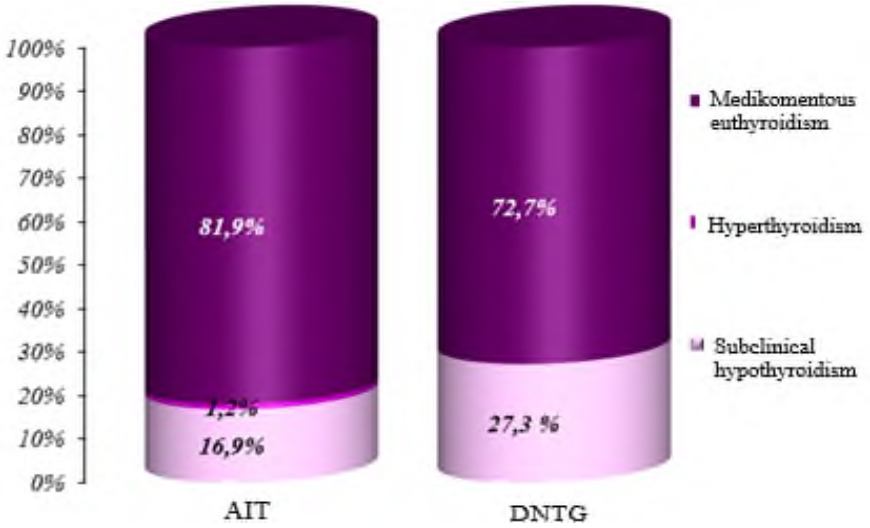
The functional status of the thyroid gland was studied in the children examined during the study.

During the evaluation of the sT3 level, a comparative analysis was conducted between the AIT group and the control group (p=0.203) and the DNTG group (p=0.187) and no statistically significant differences were found. At the same time, when evaluating the level of sT4, no statistically significant differences were revealed in the comparative analysis of its level with the group with AIT, both control (p=0.976) and group with DNTG (p=0.628).

As a result of evaluation of the TSH indicator, it was found that the median of these indicators was 1.41 mIU/L in the control group and 3.20 mIU/L in the group with AIT. If 50% of indicators in the control group were located below the interval of 1.41, this indicator was determined at the level of 3.20 in the AIT group. Which means that the boundary of 3,20 is quartile III of the control group (p<0,001).

In the study, the distribution of patients in the AIT and DNTG groups according to the functional state of the thyroid gland was carried out, and the results were presented in

Graph 1.



Graph. 1. Distribution of patients in the AIT and DNTG groups according to the functional state of the thyroid gland

According to graph 1, 68 of the children (81.9%) in the AIT group had a functional case of medicamentous euthyroidism (TSH 0.70-4.30 mIU/mL). Subclinical hypothyroidism (4,30-10 mIU/mL) functional state was detected in 14 children (16,9 %). 1 girl (1.2%) was in hyperthyroid functional state, corresponding to the thyrotoxicosis stage of AIT with clinical signs and laboratory indicators. Functional hypothyroidism was not recorded in children with AIT.

16 children (72.7%) with DNTG were in an euthyroid functional state, and 6 children (27.3%) were in a subclinical hypothyroid functional state according to laboratory indicators.

In the study, the average values of Anti-TPO antibodies in patients with AIT were 37.2 times higher than the same values in the control group, and the results were statistically significant ($p<0.001$). At the same time, when comparing the AIT group with the DNTG group, the results were also statistically significant ($p<0.001$). The

average values of Anti-TG antibodies in the AIT group were also statistically significant higher than the average values of the DNTG group ($p<0.001$). Also, the average values of the AIT group were 15.3 times higher than the average values of the control group, and the results of the statistical analysis were statistically significant ($p<0.001$). The obtained results are presented in the table 1.

Table 1.

Comparative analysis of antithyroid antibody levels

Antibodies		N	M	Std. dev.	Min	Max	Pu	Pu1
Anti-TG IU/ml	Control	15	15,1	1,3	8	24		
	AİT	83	229,6	21,2	6,83	1290	<0,001*	
	DNTG	22	25,2	1,2	16	38	<0,001*	<0,001*
Anti-TPO IU/ml	Control	15	15,0	1,1	9	22		
	AİT	83	558,5	31,1	33,59	1884	<0,001*	
	DNTG	22	36,3	2,3	23	58	<0,001*	<0,001*

Note: – the integrity of the between-group difference

P_u - with the control group

P_{u1} - with the group of AİT

*-0 hypothesis is rejected.

During the euthyroid functional state of the thyroid gland, the average levels of Anti-TPO and Anti-TG in children with AIT were significantly higher than in the control group (Anti-TPO-$p<0.001$, Anti-TG - $p<0.001$, respectively). From the results, it can be concluded that even in the euthyroid functional state of the thyroid gland, thyroid antibodies are high.

The study of the ultrasound structure of the thyroid gland in 31 children with AIT in a euthyroid functional state showed that 27 of them (87.1%) had hyperplasia of the gland (goiter), 27 (87.1%) had a decrease in the echodensity of the gland, 4 (3%) children had high vascularization, and 1 (3.2%) had fibrous septa. The results of ultrasound examinations show that changes in the structure of the thyroid gland related to autoimmune thyroid disease and hyperplasia of the thyroid gland can occur even in a euthyroid functional state.

During the study, the level of CD markers in blood was studied

in 31 children with AIT and 15 children with DNTG who were in euthyroid functional state.

The average indicators of CD3+ lymphocytes in patients with AIT were lower than the average indicators of both the control ($p<0.001$) and DNTG ($p=0.032$) groups.

A statistically significant decrease was observed in the mean indicators of CD4+ markers in the AIT group, compared to both control ($p<0.001$) and patients with DNTG ($p<0.001$).

During the study of CD8+ markers in children with AIT, their average values were statistically significantly lower than in the control group ($p<0.001$). However, the CD8+ lymphocytes in the group of children with DNTG were statistically significantly higher than in the AIT group ($p=0.002$).

The mean values of the CD4/CD8 markers in children with AIT did not change compared to the control group ($p=0.475$). There were no statistically significant changes in the mean values of CD4/CD8 markers in children with DNTG compared to children with AIT ($p=0.135$).

During the study of CD19+ lymphocytes, a 1.6-fold increase was observed in children with AIT compared to the control group, and statistically significant results were obtained ($p<0.001$). The average indicators of CD19+ lymphocytes in the group of children with DNTG were statistically significantly lower than in the AIT group ($p<0.001$).

From the results of the examinations, it is clear that changes in the immune system have occurred in children with autoimmune thyroiditis, even in the functional state of euthyroidism.

Synthesis of antibodies during the recognition of a foreign antigen in the T-cell ring is possible during the normal activity of T-helpers, but in the patients examined in our study, a decrease in the number and functional activity of T helpers was recorded.

The reduction of cytotoxic lymphocytes compared to the control group indicates the stress of cellular immunity during the chronic course of AIT.

In the study, pro-inflammatory cytokines IL-1β, IL-6 and

TNF-α involved in the pathogenesis of autoimmune thyroiditis were investigated. The obtained results are presented in the table 2.

Table 2

The cytokine status in the studied children

		N	Average indicator	Std. deviation	Min	Max	P_u	P_{u1}
TNFa pg/ml	Control	15	3,0	0,3	1,2	4,8		
	AIT	31	9,2	0,6	4,6	15,9	<0,001*	
	DNTG	14	4,2	1,0	0,3	12,6	0,896	<0,001*
IL-6 pg/ml	Control	15	2.95	0,33	1	4,8		
	AIT	31	10,90	0,82	3	19	<0,001*	
	DNTG	14	3,12	0,74	0,1	9,2	0,827	<0,001*
IL-1 pg/ml	Control	15	3,21	0,26	1,1	4,4		
	AIT	31	6,98	0,52	2,6	11,8	<0,001*	
	DNTG	14	3,78	0,81	0,1	8,6	0,776	<0,001*

Note: – the integrity of the between-group difference
P_u – with control group
*-0 hypothesis is rejected

In all children with AIT, the values of TNF-α (9.2±0.6) were practically higher compared to the control group, and the obtained results were statistically significant ($p<0.001$). When comparing the indicators of TNF-α, the level of TNF-α in the AIT group was statistically significantly higher than that of the DNTG group ($p<0.001$).

During AIT, the mean values of IL-6 were 3.7 times higher than in the control group, and the results were statistically significant ($p<0.001$).

When examining the level of IL-1β, its level increased 2.25 times in the AIT group compared to the control group, and the results were statistically significant ($p<0.001$). In spite of the tendency of IL-1β level to increase in the children with DNTG, the results compared to the control group were not statistically significant ($p=0.776$). The results are presented in the table 2.

In the study, a correlation analysis of the dependence of the level of pro-inflammatory cytokines on thyroid hormones and

antibodies in patients with AIT was performed. The results revealed a positive correlation between the level of TNF-α and the level of Anti-TPO (ρ(Rho) = 0.865*, p<0.001). The IL-6 level was in a significant negative (ρ (Rho) = -0,393*, p=0,029) correlation dependence with sT4. From this it is clear that the rise of IL-6 accelerated the inflammatory process, leading to the destruction of thyroid cells, as a result of which the synthesis of thyroid hormone decreased. Indicators of IL-1β were in direct correlation dependence with indicators of TSH hormone (ρ(Rho) =0.465*, p=0.008)

The relationship between interleukins and CD markers was investigated. It was found that there was an significant inverse correlation between TNF-α and CD4+ (ρ(Rho)=-0.744*, p<0.001) and CD8+ (ρ(Rho) =-0.461*, p<0.001) lymphocytes.

During the analysis of micrelements in the study, the average statistical value of the decreased level of selenium in the blood serum of children with AIT was 69.2±1.5 mcg/l, which was statistically significantly lower than the control group (p<0.001).No differences were found between the levels of zinc and copper between the compared groups and the control group (zinc: comparison AIT and the control group p=0.833, comparison DNTG and the control group p=0.359, copper: comparison AIT and the control group p=0.935, comparison DNTG and the control group p=0.743).

The average indicators of iodine in children with DNTG (110,7±13,9) decreased compared to the average indicators of children in the control group (195,1±11,0), and the results were statistically significant (p<0,001). In children with AIT, its average indicators (191.2±6.6) corresponded to normal indicators and there was no statistically significant difference compared to the control group (p =0.972). According to the results obtained, iodine deficiency in children with DNTG stimulated the development of goiter.

Thus, based on the results of this research work, we can note that the importance of microelements, especially selenium and iodine, for the normal functioning of the thyroid gland, is increasingly emphasized. Numerous laboratory experiments, clinical trials and epidemiological studies reveal new evidence for the role of these

microelements in the functioning of TG. It has been determined that the lack of adequate intake of some microelements in the organism can lead to a significant weakening of immunity and increased susceptibility to infections.

Studying the thyroid status, immune system indicators and the level of some microelements important for the metabolism of the thyroid gland makes it possible to analyze these important components during AIT in children and evaluate the relationship between them.

Correlation relationships between the parameters analyzed in the study were studied. The obtained results are presented in table 3.

Table 3

Correlation relationship between microelements and TSH, sT3, sT4, Anti-TPO, Anti-TG levels

Indicators		sT3	sT4	TSH	Anti-TG	Anti-TPO
Selen	ρ (Rho)	0,005	0,428*	-0,241	-0,337	-0,884*
	P	0,977	0,016	0,192	0,064	0,000
Zn	ρ (Rho)	0,035	-0,036	-0,074	-0,416*	-0,390*
	P	0,852	0,849	0,693	0,020	0,030
Cu	ρ (Rho)	-0,095	0,436*	-0,239	-0,341	-0,899*
	P	0,612	0,014	0,195	0,060	0,000
Iodine	ρ (Rho)	0,031	0,238	-0,270	-0,230	-0,516*
	P	0,869	0,198	0,142	0,212	0,003

*-0 hypothesis is rejected

As can be seen from Table 3, a positive significant correlation between selenium and sT4 indicators was found (ρ(Rho)=0.428*, p=0.016). In children with AIT, there was a negative correlation between selenium indicators and Anti-TPO (ρ(Rho)=-0.884**, p<0.001) antibodies. A negative correlation was found between iodine excretion and the level of Anti-TPO antibodies in children with AIT (ρ(Rho) =-0.516*, p=0.003).

During the interaction between the microelement of zinc and the thyroid status, a significant negative correlation relationship was

found between the average indicators of zinc and the indicators of anti-TQ and anti TPO antibodies (respectively, Anti-TG ρ(Rho) = -0,416*, p=0,020, Anti-TPO ρ(Rho) = -0,390*, p=0,030).

Positive significant correlation relationship between copper and sT4 (ρ(Rho) =0.436, p=0.014) has been detected. There was a negative correlation relationship of indicators of copper in children with AIT with indicators of Anti-TPO (ρ(Rho) =-0,899**, p<0,001) antibodies.

In the course of the study, the correlation relationship between microelements and indicators of cellular immunity in children with AIT was studied.

In children with AIT, a rather strong inverse correlation was found between the level of TNF-α and the level of selenium (ρ(Rho) =-0.940*, p<0.001). Thus, the statistical analyzes carried out showed that regardless of whether patients with AIT are in the functional status of medicamentous euthyroidism, the deficiency of the selenium microelement indicates an increase in the level of inflammatory-directed cytokines in them.

A negative correlation was found between the level of iodine in urine and the level of TNF-α (ρ(Rho) =-0.674, p<0.001), which indicates that maintaining an adequate level of iodine in children will promote the normal functioning of the immune system.

In the second part of the study, 31 euthyroid functional AIT patients with an average age of 11.16 ± 0.59 were included and they were divided into two groups. The first group included 14 children (average age 10.5±0.92). The ultrasound examination of the TG is characterized by hyperplasia of the gland and a decrease in echodensity. Children from this group were in a euthyroid status after receiving L-thyroxine treatment in individual doses.

The second group included 17 children diagnosed with AIT (average age 11.52±0.74). During treatment, the euthyroid functional status of the thyroid gland was preserved against the background of replacement therapy in patients in both groups. In addition to the main (replacement) treatment with L-thyroxine, selenium (selenium 110 mcg) was prescribed to patients from this group in a dose of 1

capsule once a day for 6 months. The indication for determining selenium was the low level of selenium in the blood serum of the patients. During the ultrasound examination of the thyroid gland, hyperplasia of the gland was recorded in 15 children, and a decrease in echodensity in 15 children.

Catamnestically, the level of Anti-TPO was assessed in both compared subgroups after the completion of the 6-month follow-up period. At the beginning of the follow-up period, the average indicators of Anti-TPO were approximately similar in both subgroups, statistically significant did not differ ($p=0.258$). At the end of the follow-up period, although there was a tendency to decrease the average indicators of Anti-TPO in subgroup I, which received replacement treatment with L-thyroxine, these results were not statistically significant compared to the beginning of the examination ($p=0.594$).

At the end of the 6-month follow-up period, the level of Anti-TPO in the II subgroup decreased significantly compared to the beginning of the follow-up period ($p=0.075$), but it was still high compared to the control group ($p<0.001$).

During the observation, although there was a trend of decrease in the average indicators of Anti-TG in subgroup I, these results were not statistically significant ($p=0.124$), and were 14 times higher than the control group ($p<0.001$). After the 6-month follow-up period in the second subgroup receiving selenium, no significant differences were found in the indicators of Anti-TG ($p=0.075$). At the end of the follow-up period, the level of Anti-TG in the II subgroup was statistically higher compared to the control group ($p<0.001$).

At the beginning of the follow-up period during the study, the levels of TSH, sT4 and sT3 hormones in both subgroups did not have statistical differences before treatment. At the end of the follow-up period, although there was a trend of decrease in the average indicators of TSH in subgroup I, the results were not statistically significant ($p=0.594$). After the end of the follow-up period, the average indicators of TSH in the II subgroup decreased compared to the previous indicators, and the obtained

results were statistically significant (p=0.032). A similar trend was observed with thyroid hormones.

In subgroup I, the mean values of sT4 were 1.20±0.08 mIU/ml before treatment, but after the end of the follow-up, they increased to 1.31±0.08 mIU/ml, and these results were statistically significant (p=0.038). In the second subgroup, there was an increase in the average indicators of sT4 compared to the indicators before the treatment, and the obtained results were statistically significant (p=0.021).

In subgroup I, an increase was observed in the average indicators of sT3 after 6-month follow-up period, and the results were statistically significant (p=0.041). In the second subgroup, the average values of sT3 were 2.83±0.13 pg/ml before the treatment, and after the end of the follow-up, they increased to 3.36±0.14 pg/ml, and these results were statistically significant (p<0.001).

Thus, considering our results, increasing the level of selenium in the blood serum has an inhibitory effect on the activity of thyroid antibodies by lowering them.

At the end of the 6-month follow-up period, no change in the level of selenium was detected in the I subgroup (p<0.001 compared to the control group), but the average indicators of selenium in all the children in the II subgroup significant increased compared to the indicators at the beginning of the study (p<0.001). No statistically significant changes were recorded in the titers of other microelements in either half-group.

The total number and dynamics of CD3+, CD4+, CD8+, CD4+/CD8+, CD19+ in children from both groups were studied and analyzed.

T-cell suppression was detected before treatment in children examined in both subgroups. In subgroup I, a statistically significant increase was noted in the average indicators of CD3+ lymphocytes after 6 months of follow-up (p=0.001). The presence of positive dynamics in this group is explained by the fact that thyroxine and triiodothyronine have an immunomodulating effect. After the end of the 6-month follow-up period, an

significant increase of CD3+ lymphocytes ($p<0.001$) was noticed in subgroup II.

Despite the fact that there was a trend of increase in the mean indicators of CD4+ lymphocytes after the end of the follow-up period in subgroup I patients, significant differences were not detected ($p=0.431$). The number of CD4+ lymphocytes in the children of the II subgroup significant increased at the end of the follow-up period ($p=0.001$). A similar situation was repeated in CD8+ lymphocytes during dynamic analysis. If the significant increase in CD8+ lymphocytes was not observed in patients in subgroup I ($p=0.480$), the number of CD8+ lymphocytes increased significantly in subgroup II after the follow-up period ($p=0.001$). Although the average indicators of CD 19+ lymphocytes decreased after treatment in both subgroups, the increase in these indicators was statistically significant in subgroup II ($p<0.001$).

The level of TNF-α, IL-6 and IL-1β in both comparison subgroups was studied and analyzed.

Before the 6-month follow-up, the mean levels of TNF-α and IL-6 were almost 2 times higher than the normal values at this age and were not statistically different before treatment in both subgroups (respectively, TNF-α: p=0,977, IL-6: p =0,592).

After the end of the follow-up period, although a tendency to decrease in the average indicators of TNF-α was observed in subgroup I, this difference was not statistically significant ($p=0.124$). In subgroup II, a decrease in TNF-α average indicators was observed, and the difference was statistically significant ($p=0.028$).

Although there was a decrease in the mean values of IL-6 in subgroup I after the end of the follow-up period, the difference was not statistically significant ($p=0.485$). In the second subgroup, a decrease in IL-6 average indicators was observed, and the difference compared to the previous indicators was statistically significant ($p=0.002$).

After the end of the follow-up period, IL-1β indicators in subgroup I showed a tendency to decrease compared to the previous indicators, but this difference was not statistically significant ($p=0.141$). However, after the end of the follow-up

period, the mean values of IL-1β in subgroup II decreased slightly, and this difference was statistically significant compared to the values before the follow-up period (p=0.009). The obtained results are presented in the table 4.

Table 4

Comparative analysis of cytokine concentrations before and after treatment

Indicators pq/ml	I group (n=14)					II group (n=17)				
	M	Me	Q1	Q3	Pw	M	Me	Q1	Q3	Pw
TNF-α/ after	9,16	8,85	6,60	11,50	0,124	9,31	8,60	6,80	12,10	0,028*
TNF-α/ before	7,03	7,10	4,70	9,30		6,54	6,40	4,40	7,80	
IL-6 / after	10,43	9,10	7,60	15,40	0,485	11,29	12,90	8,50	14,80	0,002*
IL-6/ before	8,66	8,95	6,20	11,20		6,34	5,60	4,80	8,50	
IL-1 β/ after	6,79	6,85	4,50	9,10	0,141	7,15	8,60	3,70	9,40	0,009*
IL-1 β/ before	6,69	6,55	4,40	9,00		5,15	4,00	3,60	5,80	

Note: – the integrity of the between-group difference

P_w – comparison of subgroups before and after treatment

*- 0 hypothesis is rejected

During the study, according to the results of the ultrasound examination of the thyroid gland, there was no statistically significant decrease in thyroid hyperplasia during the 6-month follow-up period in subgroup I (p=0.317). In subgroup II, a statistically significant decrease in these indicators was observed after the end of the follow-up period (p=0.025).

After the follow-up period, there was no decrease in the number of children with a decrease in thyroid gland echodensity in subgroup I, and the differences were not significant (p=0.317). A decrease in the number of children with a decrease in thyroid gland exodensity in the II subgroup was observed after the follow-up period, and the obtained differences were statistically significant (p=0.046).

Thus, based on these results, it can be said that by lowering the level of thyroid antibodies, selenium also lowered the level of pro-

inflammatory cytokines and had a positive effect on the ultrasound structure of the thyroid gland.

In parallel with the conducted research, the quality of life of the children was evaluated based on the anamnestic data obtained from the children and their parents before and 6 months after the treatment. After the 6-month follow-up period, there was no statistically significant decrease in subgroup I (improvement of sleep pattern p=0.157, improvement of mood p=0.083, improvement of memory p=0.157, respectively).

At this time, the parents of the children in the subgroup II reported that the children's mood improved significantly. From the obtained results, it was revealed that in the second subgroup, statistically significant results were obtained in the investigated clinical signs (improvement of sleep mode (p=0.008), improvement of mood (p=0.008), improvement of memory (p=0.008)).

From the results obtained during the research, it can be concluded that selenium also had a positive effect on the quality of life of children. Our results show that the intake of selenium in selenium-deficient children can have a positive effect on improving the quality of life of patients.

Thus, the studied research work showed that the additional intake of this important microelement in case of selenium deficiency has a significant effect on inflammatory activity in case of autoimmune diseases of the thyroid gland. Our results indicate the effectiveness of the use of Selenium (selenium 110 mcg) in combination therapy during the treatment of autoimmune thyroiditis. Taking into account the results of these and other research works, it can be concluded that for patients with autoimmune thyroiditis, selenium can be useful in reducing the level of the inflammatory process and antibodies, improving the ultrasound structure of the thyroid gland, and improving the quality of life of patients. In connection with the above, it is recommended to use selenium in the complex treatment of autoimmune thyroiditis.

RESULTS

1. 81.9% of children with autoimmune thyroiditis had drug-induced euthyroidism, 16.9% of children had subclinical hypothyroidism, and 1.2% of children had functional hyperthyroidism. In children with AIT, the average indicators of TSH were 1.7 times higher than the control group (p<0,001), while the average indicators of sT4 (p=0,976) and sT3 (p=0.203) did not differ. The average indicators of anti-TPO (37 times, p<0,001) and anti-TG (15 times, p<0,001) antibodies were statistically significantly higher in the euthyroid functional state of the thyroid gland compared to the control group [10,12,13].

2. Among children with AIT with high TSH and thyroid antibodies, 21 (25.3%) had a feeling of suffocation (p_x2=0,060), 41 (49.4%) had a sleep pattern disorders (p_x2=0,001), 45 (54.2 %) – mood disorders (p_x2<0,001), 38 (45.7%) - memory disorders (p_x2=0,004) and 11 (13.3%) had hair loss (p_x2=0.021) [2].

3. In patients with AIT, the level of CD3+ (p<0,001), CD4+ (p<0,001), CD8+ (p<0,001) markers decreased compared to the control group, CD19+ (p<0,001) markers 64, 5% increase was observed. An increase in the level of IL-1β interleukin in the blood serum by 2.17 times (p<0,001), IL-6 by 3.7 times (p<0,001), and TNF-α by 3 times (p<0,001), statistically significant, affects the acceleration of the synthesis of thyroid antibodies, causing an increase in CD19+ markers [10,11,13].

4. In children with AIT, a decrease in the average indicators of selenium was observed, and in comparison with the control group, it was significantly low (p<0,001), which can be assessed as one of the reasons for the destruction of the thyroid gland. Significant differences in the level of zinc (p=0,833), copper (p=0,935) and iodine (p=0,972) were not found [6,7,10].

5. Indicators of selenium in the blood serum of children with autoimmune thyroiditis have a strong negative correlation with anti-TPO antibodies (ρ=-0.884**, p<0.001) and the level of TNF-α interleukin (ρ =-0.940**, p<0.001) and an increase in the immune response was observed at this time. A significant negative correlation (c=-0.516**, p=0.003) was found between iodine excretion and the level of anti-TPO antibody [12,14].

6. The appointment of the drug selenium for 6 months at a dose of 110 mcg per day was accompanied by clinical improvement in children with AIT, leading to a decrease in the average indicators of anti-TPO antibodies (p=0,044), TSH hormone (p=0,032), TNF-α (p=0,028), IL-6 (p=0,002), IL-1β (p=0,009) interleukin in the blood serum [1,3,4,5].

PRACTICAL RECOMMENDATIONS

1. In children with autoimmune thyroiditis, it is recommended to include IL-6 cytokine levels determitaion in the examination complex, regardless of the functional status of the thyroid gland.

2. In children with autoimmune thyroiditis, regardless of the functional status of the thyroid gland, it is recommended to include in the complex of examinations, the determination of the level of selenium in the blood serum.

3. Taking into account the effectiveness of the study and in order to restore the microelement composition of the blood in children diagnosed with autoimmune thyroiditis, it is recommended to add selenium to complex therapy at age-appropriate dosagetimes a day for 6 months.

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LIST OF ABBREVIATIONS

AIT	–	autoimmune thyroiditis
Anti-TPO	–	antibodies against thyroid peroxidase
Anti-TG	–	antibodies against thyroglobulin
DNTG	–	diffuse non-toxic goiter
MHC	–	major histocompatibility complex
TSH	–	thyroid stimulating hormone
T4	–	tiroxin
T3	–	triiodothyreonine
TG	–	thyroglobulin
TPO	–	thyroid peroxidase
TG	–	thyroid gland
IgE	–	immunoglobulin E
IgA	–	immunoglobulin A
IgM	–	immunoglobulin M
IgG	–	immunoglobulin G
IL	–	interleukin
TNF	–	tumor necrosis factor
GPx	–	glutathione peroxidase

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