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ORIGINAL PAPER

CASPASE-DEPENDENT MECHANISMS AND ANTIOXIDANT DEFENSE IN THE THYROID TISSUE OF PATIENTS WITH NODULAR GOITER WITH AUTOIMMUNE THYROIDITIS AND THYROID ADENOMA ACCORDING TO ALLELIC STATUS OF *BCL-2 (RS17759659), CTLA-4 (RS231775), APO-1 / FAS (RS2234767)* GENES

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Abstract

Introduction. In the process of their vital activity, the cells are exposed to many damaging factors of endogenous and exogenous nature. It is undoubtedly that various toxic influences or metabolic disorders lead to the development of oxidative stress, and in this case the future of the cell is determined by a balance of various adaptive metabolic processes induced by a pathological factor, as well as by genetic and constitutional features of its biochemical systems.

Résumé

Les mécanismes dépendant de caspases et la protection antioxydante du tissu thyroïdien des patients avec goître nodulaire associé à la thyroïdite auto-immune et au adénome thyroïdien par rapport au statut des gènes allèles Bcl-2, CTLA-4, APO-1/Fas

Introduction. Dans le processus de leur activité vitale, les cellules sont exposées à de nombreux facteurs néfastes de nature endogène et exogène. Il est sans aucun doute que diverses influences toxiques ou troubles

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Objectives. To analyze the mechanisms of caspase-dependent apoptosis and pro- and antioxidant activity in the thyroid tissue homogenate in patients with NGAIT and TA considering the polymorphic variants of BCL-2 (rs17759659), CTLA-4 (rs231775), APO-1 / Fas (rs2234767) genes.
Methods. We investigated pro- and antioxidant activity and the activity of caspases 3 and 8 in 5% of thyroid tissue homogenates. The BCL-2 (rs17759659), CTLA-4 (rs231775), Fas (rs2234767) genes polymorphism was studied by Real-Time Polymerase Chain Reaction in 95 patients with NGAIT, 30 patients with TA and 25 healthy individuals.
Results. In homozygous carriers of A-allele of the BCL-2 (rs17759659) gene, the activity of effector caspase-3 is higher than in the control group.
Conclusions. The imbalance between the activity of peroxidation and antioxidant defense in patients with NGAIT and TA is associated with the promoter of the CTLA-4 (rs231775) and APO-1 / Fas (rs2234767) genes and is characterized by an increasing degree of OMP in the altered thyroid tissue and also with a lower capacity of the AOP system enzymes.

Key words: goiter, thyroiditis, caspases, oxidant status, genetic association.

Abbreviations: NGAIT – nodular goiter on the background of autoimmune thyroiditis; TG – thyroid gland, TA – thyroid adenoma, OMP – oxidative modification of proteins, AOP – antioxidant protection.

Objectifs. Analyser les mécanismes de l'apoptose dépendante de caspases et de l'activité pro et antioxydante dans l'homogénéisation du tissu thyroïdien chez les patients atteints de GNTAI et AT en considérant les variantes polymorphes de gènes BCL-2 (rs17759659), CTLA-4 (rs231775), APO-1 / Fas (rs2234767).
Méthodes. En outre, nous avons étudié l'activité pro et antioxydante et l'activité des caspases 3 et 8 dans 5% des homogénats du tissu thyroïdien. Le polymorphisme des gènes BCL-2 (rs17759659), CTLA-4 (rs231775), Fas (rs2234767) a été étudié par réaction en chaîne en polymères en temps réel chez 95 patients atteints de GNTAI, 30 patients atteints de TA et 25 personnes en bonne santé.
Résultats. On doit noter que dans les supports homozygotes de l'allèle A du gène BCL-2 (rs17759659), l'activité de la caspase-3 effectrice est supérieure à celle du groupe témoin.
Conclusions: Le déséquilibre entre l'activité de la peroxydation et la défense antioxydante chez les patients atteints de GNTAI et AT est associé au promoteur des gènes CTLA-4 (rs231775) et APO-1 / Fas (rs2234767) et est caractérisé par un degré croissant de MOP dans le tissu thyroïdien altéré avec une moindre capacité des enzymes du système POA.

Mots clés: goitre, thyroïdite, caspases, statut oxydant, association génétique.

Abréviations: GNTAI – goître nodulaire au fond de la thyroïdite auto-immune; GT – glande thyroïde, AT – adénome thyroïdien, MOP – modification oxydante des protéines, POA – protection antioxydante.

Introduction

Autoimmune thyroiditis (AIT) is the most common thyroid disease and accounts for 46% of all thyroid diseases[1,2].

The incidence of AIT increases constantly, and in the following years it is expected to maintain a growing trend[1-3]. Increasingly, after thyroid gland surgery, nodes are diagnosed with AIT. There are difficulties of diagnosing the AIT and nodes in patients with AIT in the preoperative period. Concomitant thyroiditis considerably complicates the diagnosis of nodal goiter[4].

Metabolic disorders and accumulation of various toxic substances in the body eventually lead to oxidative stress, under which the cell fate is determined by the aggregate balance of the adaptive processes, as well as by genetic and constitutional features of its biochemical systems[5-9]. Under these circumstances, oxidation of lipids and proteins of the cell membrane, under the influence of excessive production of reactive oxygen species, is an early stage of the cellular apoptosis[10-13]. However, the implementation of apoptosis, the mechanisms of its induction and regulation depend on a number of reasons: the activity of mitochondrial systems of the cell power supply (inner apoptosis way via activation of caspase 9)[14,15], certain molecular genetic factors encoding the work of enzymes[16], immune homeostasis (external

métaboliques conduisent au développement du stress oxydatif, et dans ce cas, l'avenir de la cellule est déterminé par un équilibre de divers processus métaboliques adaptatifs induits par un facteur pathologique ainsi que par des caractéristiques génétiques et constitutionnelles de ses systèmes biochimiques.

way of apoptosis – by the activation of Fas-receptors (CD95) and caspase 8), the activity of peroxidation processes[14-17]. Both ways lead to the activation of the effector caspase-executors of apoptosis (caspase 3, 6, 7), which results in DNA degradation and cell death[18,19].

Due to the above, the purpose of our study was to assess the mechanisms of caspase-dependent apoptosis and pro- and antioxidant activity in the thyroid tissue homogenate, in patients with AIT and TA, considering the polymorphic variants of BCL-2 (rs17759659), CTLA-4 (rs231775), APO-1 / Fas (rs2234767) genes.

Material and methods

95 women with NGAIT were examined between 2013- 2016 in Chernivtsi regional clinical hospital. The age of patients ranged from 23 to 72 years. The diagnosis was made clinically, in laboratory (thyroid peroxidase antibodies (TPAB) – 60-250 U/ml, thyroglobulin antibodies (TGAB) – 60-500 U/ml, thyroid-stimulating hormone (TSH) – 4.10 mU/L), using ultrasound, and it was confirmed histologically after the surgery.

We selected a group of 30 women who had been diagnosed with thyroid adenoma after surgery, ultrasonography, fine-needle aspiration biopsy (FNAB) and histological conclusion. 25 healthy donors were examined, as well. We used for the study 10 ml of peripheral blood drawn from the ulnar vein in the morning, on an empty stomach.

All the patients underwent surgery, from hemithyroidectomy to thyroidectomy. The tissue has been taken from the operating room no later than 30 minutes after surgery. In patients from the 1st group, we isolated separately macroscopically unchanged tissue (paranodular), which served as control for both compared groups, and adenomatous tissue. In patients with AIT, we took the tissue from the left, right lobes and from the isthmus.

The pieces of tissue, weighing 100-300 mg, were transported to the laboratory on ice and immediately cut into 4-6 pieces, weighing an average 50-70 mg each. After the partition, they were closed in a special plastic container and stored at –70°C before tests. We investigated pro- and antioxidant activity in 5% of thyroid tissue homogenates, by determining the activity of glutathione peroxidase (GP mmol/min • g tissue), glutathione-S-transferase (GST, umol/min • g tissue) and degree of oxidative changes of proteins (OMP, optical density unit/g protein) by accepted methods.

To study the activity of caspases 3 and 8, we crushed the thyroid tissue in homogenizer «WiseTis» HG-15 series (Daihan Scientific, South Korea) with a rotor 8 mm at a speed of 4500 rev/min. We used the isolating medium (20 mM HEPES, pH 7.5, 10 mM KCl, 1.5 mM MgCl2, 1 mM DTT), to which a cocktail of protease inhibitors (104 mM AEBSF, 0,08 mM aprotinin, 1.5 A pepstatin mM, 2 mM leptin 4 bestatin mM, 1.4 mM E-64) was added at a ratio of 100:1 (all reagents were manufactured by Sigma, USA). The homogenates were centrifuged at microcentrifuge Heraeus fresco 17 (Thermo Electron LED GmbH, Germany) at 1500 revolutions for 30 minutes, at a temperature of +4 ° C.

The resulting supernatant was used to assess the activity of caspase-3 and caspase-8. Specific activity of the effectors' caspase-3 and initiator caspase-8 in the tissue was studied, using colorimetric method with enzyme-linked immunoabsorbent (ELISA) Sanrise™-Tecan (Austria), at a wavelength of 405 nm, with the speed of splitting the synthetic substrate Nacetyl-Asp-Glu – Val-Asp-nitroanilin (Ac DEVD-NHA) and N-acetyl-Ili-Glu-Asp-Trenitroaniline (Al-IETD-PNA) respectively. All reagents used in this study were made by the company Sigma (USA). Caspase activity was assessed in (mmol of paranitro-aniline/ [h • mg of protein]).

The DNA was isolated by a set of reagents Thermo Scientific Gene JET Genomic DNA Purification kit (# K0721, Thermo Fisher Scientific), with incubation with proteinase K overnight, to complete cell lysis. The purified DNA was diluted in Elution Buffer and evaluated with a spectrophotometer Nanodrop2000C. Only samples with a concentration not lower than 15 ng / ml and the values of the ratio of A (260/280) between 1.7 and 2.0 were used for genotyping. The obtained extracts were divided into aliquots, one of which was placed in a refrigerator at 4°C until its use, while others were frozen at –20°C.

To normalize the amount of DNA, all samples were brought to a concentration of 2 ng/μL using nuclease-free water.

To genotype the selected point polymorphism, the TaqMan technology was used. Polymorphisms marked with the reference number SNP ID, according to the database dbSNP have been studied. To test each of the polymorphisms TaqMan® SN Genotyping Assays (40X) (4,351,379, Thermo Fisher Scientific) were used (Table 1)

The volume of the reaction mixture was 5 μL and consisted of: 2.5 μL reagent Taq Man Genotyping Master Mix (20X) (4,371,355, Thermo Fisher Scientific), 0,25 μL of probe solution and 2.25 μL of DNA solution. Genotyping was performed with the instrument Quant Studio 6 (Applied Biosystems, Thermo Fisher Scientific), 384-well block.

Table 1. The nucleotide sequence of the region including the analyzed polymorphism.

Reference number SNP ID	Test number (Assay ID*)	Fragment of the region including the analyzed polymorphism
rs231775 (CTLA4)	C___*2415786_20*	GCACAAGGCTCAGCTGAACCTGGCT[**A/G**] CCAGGACCTGGCCCTGCACTCTCCT
rs17759659 (BCL2)	C__*33628167_10*	TCTTCTTACCAAAGATTCACAATAC[**A/G**] GTGTTGATGGGAACGTGACCTAGTT
rs2234767 (FAS)	C__*12123966_10*	CAGAGTGTGTGCACAAGGCTGGCAC[**A/G**] CCCAGGGTCTTCCTCATGGCACTAA

Note: according to the website www.thermofisher.com.

Amplification was performed under the following conditions:

Activation	10 min	95°C	
Denaturation	15 sec	92°C	40*/60** cycles
Annealing / elongation	1 min	60°C	

Note: * – to amplify the polymorphisms associated with CTLA-4 and Fas genes; ** – to amplify the polymorphisms associated with Bcl-2 genes.

To collect and process the data, the software *Quant Studio™ Real-Time PCR* (v.1.3) was used.

The main part of the statistical analysis was carried out using the software Statistica 7.0 (SPSS). Nominal data are presented in the form of quantitative values and percentages. The balance Hardy – Weinberg of the genotype distribution was checked using Online Encyclopedia for Genetic Epidemiology Studies (http://www.oege.org/software/hwe-mr-calc.shtml). To compare the distribution of genotypes in the experimental and control groups, Pearson's chi-squared test was used. The reliability of differences of averages in groups with different genotypes was determined by the method of univariate analysis of variance (ANOVA). The impact of factors on the development of thyroid pathology was assessed using a binary logistic regression model for the relative risk (RelR), risk ratio (RR) and odds ratio (OR) with 95% confidence interval [95% CI], taking into account the criterion χ^2 (df = 1). The difference was considered reliable at $p < 0.05$.

Results and discussion

The degree of protein oxidation and antioxidant defense, as well as the activity of caspases –3 and –8 in the altered thyroid tissue, considering polymorphic variants of the BCL-2 (rs17759659) gene, are shown in Table 2. In the thyroid tissue, there was an

Table 2. The activity of caspases –3 and-8, of protein peroxidation and antioxidant defense in the thyroid tissue considering the polymorphic variants of the BCL-2 (rs17759659) gene.

Indices	Control (morphologically unaltered thyroid tissue), n=25	Genotypes of the *BCL-2* gene in patients		
		AA, n=10	AG, n=110	GG, n=5
Degree of OMP, **optical density unit**/g of protein	46.51±0.46	64.18±1.95 p<0.001	63.02±2.64 p<0.001	64.92±1.97 p<0.001
Activity of GP mmol/min • g tissue	191.65±1.48	150.21±4.73 p<0.001	154.14±6.67 p=0.002	148.71±4.50 p<0.001
Activity of GST, mmol/min • g tissue	24.48±0.92	13.57±1.31 p<0.001	13.35±1.65 p<0.001	13.08±1.47 p<0.001
Caspase-3, mmol of para nitroaniline/[h • mg of protein]	0.098+0.007	0.167±0.033 p=0,049	0.148±0.036	0.153±0.038
Caspase-8, mmol of para nitroaniline/[h • mg of protein]	0.993±0.062	1.304±0.054 p=0.006	1.244±0.069 p=0.008	1.353±0.052 p=0.004

Notes: 1. OMP – oxidative modification of proteins; GP – glutathione peroxidase; GST – glutathione-S-transferase; TG – thyroid gland; 2. p – reliability of index differences compared to control group; p_{AA} – reliability of index differences compared to the carriers of AA-genotype; p_{AG} – reliability of index differences compared to the carriers of AG-genotype.

Table 3. Indices of oxidative status, antioxidant defense and the activity of caspases-3 and 8 in the thyroid tissue considering the polymorphic variants of the BCL-2 (rs17759659) gene.

Indices	Changes in indices, n	Genotypes of *BCL-2* gene, n=125 (%)		
		AA, n=10	AG, n=110	GG, n=5
Degree of OMP	Moderate increase (≤50 percentiles), n=30	1 (10.0)	29 (26.36)	0
	Significant increase (>50 percentiles), n=95	9 (90.0)	81 (73.64)	5 (100.0)
χ^2; p		χ^2=12.80 p<0.001	χ^2=49.16 p<0.001	-
GP and GST activity	Moderate decrease (≤50 percentiles), n=30	1 (10.0)	29 (26.36)	0
	Significant decrease (>50 percentiles), n=95	9 (90.0)	81 (73.64)	5 (100.0)
χ^2; p		χ^2=12.80 p<0.001	χ^2=49.16 p<0.001	-
Caspase-3, Caspase-8, un/ml	Moderate increase (≤50 percentiles), n=30	1 (10.0)	29 (26.36)	0
	Significant increase (>50 percentiles), n=95	9 (90.0)	81 (73.64)	5 (100.0)
χ^2; p		χ^2=12.80 p<0.001	χ^2=49.16 p<0.001	-

increase of parameters of oxidative modification of proteins (OMP) by 35.50-39.58% (p <0.001), against the background of lower activity of antioxidant defense (AOP) enzymes- glutathione peroxidase (GP) (by 19.57- 22.41%, p≤0,002) and glutathione-S-transferase (GST) (by 44.57-46.57%, p <0.001). We have not established any clear dependence of OMP and AOP on the polymorphism of the BCL-2 (rs17759659) gene.

The activity of caspase-8 (Table 2) increases in the thyroid tissue, regardless of the genotypes of the *BCL-2* (*rs*17759659) gene, by 25.28-36.25% (p≤0,008). At the same time, the activity of the effector caspase-3 increases statistically significant only in the homozygous carriers of *A*-allele by 70,41% (p=0.049).

Univariate analysis of variance confirmed the association of the promoter of *BCL-2* (*rs*17759659) gene with the index of the activity of the antioxidant defense system (F=3.17, p=0.046), as well as with caspase-8, which implements the external Fas-dependent way of apoptosis (F=9.32, p<0.001) (Table 2).

There were more patients with thyroid pathology and increased degree of OMP and the activity of caspases –3 and –8 in thyroid tissue homogenate, against the background of significant AOP reduction (> 50 percentiles) than those with a moderate increase (≤50 percentiles) of OMP and caspases and a decrease in the activity of AOP by 2.79-9 times (p <0.001), respectively, without reliable differences between the genotypes of the BCL-2 (rs17759659) gene. (Table 3).

The degree of OMP in the thyroid tissue was higher in patients with *A*- allele of the *CTLA-4* (*rs*231775) gene in the genotype (Table 4) than in the homozygous carriers of the minor G-allele by 29.37% (p_{AA}=0.005) and 32.24% (p_{AG}=0.003), against the background of lower activity of AOP enzymes: by 14.0% (p_{AA}=0.009) and 15.16% (p_{AG}=0.005) than GP, by 34.31% (p_{AA}=0.009) and 39.83% (p_{AG}=0.004) than GST, respectively. A similar trend was observed in the activity of caspase-8, which prevailed in the carriers of wild A-allele of the CTLA-4 (rs231775) gene, over the index of individuals with GG-genotype by 13.56% (p_{AA}=0,03) and 12.84% (p_{AG}=0.041). The activity of caspase-3 between the observation groups did not differ statistically significant. (Table 4).

Univariate analysis of variance confirmed the association of promoter of the CTLA-4 (rs231775) gene with the oxidative stress – OMP (F=116.41, p<0.001), the activity of the antioxidant stress system in GP (F=52.36, p<0.001) and GST (F=91.37, p<0.001), as well as the marker of apoptosis caspase-8, (F=16.0, p<0.001) (Table 4).

There were more patients with thyroid pathology and increased degree of OMP and the activity of caspases –3 and –8 in thyroid tissue homogenate, against the background of significant AOP reduction (> 50 percentiles), than those with a moderate increase (≤50 percentiles) of OMP and caspases and a decrease in the activity of AOP by 2.44-3.92 times (p <0.001), respectively, without significant differences between the genotypes of the *CTLA-4* (*rs*231775) gene (Table 5).

Table 4. The activity of caspases-3 and –8, protein peroxidation and antioxidant defense in the thyroid tissue, considering the polymorphic variants of the CTLA-4 (rs231775) gene.

Indices	Control (morphologically unaltered thyroid tissue), n=25	Genotypes of the *CTLA-4* gene in patients		
		AA, n=59	AG, n=62	GG, n=4
Degree of OMP, **optical density unit**/g of protein	46.51±0.46	62.59±2.18 p<0.001	63.98±1.73 p<0.001	48.38±2.84 p_{AA}=0.005 p_{AG}=0.003
Activity of GP, mmol/min • g tissue	191.65±1.48	154.59±5.80 p=0.001	152.50±4.50 p<0.001	179.75±4.88 p=0.028 p_{AA}=0.009 p_{AG}=0.005
Activity of GST, mmol/min • g tissue	24.48±0.92	13.92±1.41 p=0.001	12.75±1.03 p<0.001	21.19±1.60 p_{AA}=0.009 p_{AG}=0.004
Caspase-3, mmol of para nitroaniline/[h • mg of protein]	0.098+0.007	0.152±0.038	0.144±0.033	0.126±0.012
Caspase-8, mmol of para nitroaniline/[h • mg of protein]	0.993±0.062	1.256±0.051 p=0.01	1.248±0.052 p=0.002	1.106±0.044 p_{AA}=0.03 p_{AG}=0.041

Notes: 1. OMP – oxidative modification of proteins; GP – glutathione peroxidase; GST – glutathione-S-transferase; TG – thyroid gland; 2. p – reliability of index differences compared to control group; p_{AA} – reliability of index differences compared to the carriers of AA-genotype; p_{AG} – reliability of index differences compared to the carriers of AG-genotype.

Table 5. Indices of oxidative status, antioxidant defense and the activity of caspases-3 and 8 in the thyroid tissue, considering the polymorphic variants of the CTLA-4 (rs231775) gene.

Indices	Changes in indices, n	Genotypes of *CTLA-4*gene, n=125 (%)		
		AA, n=59	AG, n=62	GG, n=4
Degree of OMP	Moderate increase (≤50 percentiles), n=30	12 (20.34)	18 (29.03)	0
	Significant increase (>50 percentiles), n=95	47 (79.66)	44 (70.97)	4 (100.0)
χ^2; p		χ^2=41.53 p<0.001	χ^2=21.81 p<0.001	-
GP and GST activity	Moderate decrease (≤50 percentiles), n=30	12 (20.34)	18 (29.03)	0
	Significant decrease (>50 percentiles), n=95	47 (79.66)	44 (70.97)	4 (100.0)
χ^2; p		χ^2=41.53 p<0.001	χ^2=21.81 p<0.001	-
Caspase-3, Caspase-8, un/ml	Moderate increase (≤50 percentiles), n=30	12 (20.34)	18 (29.03)	0
	Significant increase (>50 percentiles), n=95	47 (79.66)	44 (70.97)	4 (100.0)
χ^2; p		χ^2=41.53 p<0.001	χ^2=21.81 p<0.001	-

The degree of OMP in the altered thyroid tissue in patients with AIT and TA, as well as the activity of caspases –3 and –8, exceeded the performance in the control group, regardless of the genotype of *APO-1/Fas* (*rs2234767*) gene (Table 6): that of OMP by 33.39% and 36.06% (p<0.001), that of caspase-3 by 41.84% (p=0.007) and 54.08% (p=0.009), caspase-8 – by 23.46% (p=0.008) and 26.49% (p=0.01), respectively. Against this backdrop the activity of AOP enzymes decreased, which did not depend on the polymorphic variants of the APO-1 / Fas (rs2234767) gene: by 18.37% (p = 0.001) and 19.93% (P <0.005) of that of GP, by 42.48% (p = 0.001) and 45.59% (p <0.001) of GST, respectively (Table 6).

Univariate analysis of variance confirmed the association of promoter of the *APO-1/Fas* (*rs2234767*)

Table 6. The activity of caspases-3 and –8, protein peroxidation and antioxidant defense in the thyroid tissue considering the polymorphic variants of the APO-1/Fas (rs2234767) gene.

Indices	Control (morphologically unaltered thyroid tissue), n=25	Genotypes of *Fas* gene in patients	
		AG, n=23	GG, n=102
Degree of OMP, **optical density unit**/g of protein	46.51±0.46	62.04±2.19 p<0.001	63.28±1.96 p<0.001
Activity of GP, mmol/ min • g tissue	191.65±1.48	156.44±5.28 p=0.001	153.45±4.85 p<0.001
Activity of GST, mmol/ min • g tissue	24.48±0.92	14.08±1.40 p=0.001	13.32±1.23 p<0.001
Caspase-3, mmol of para nitroaniline/ [h • mg of protein]	0.098+0.007	0.139±0.009 p=0.007	0.151±0.014 p=0.009
Caspase-8, mmol of para nitroaniline/ [h • mg of protein]	0.993±0.062	1.226±0.055 p=0.008	1.256±0.051 p=0.01

Notes: 1. OMP – oxidative modification of proteins; GP – glutathione peroxidase; GST – Glutathione-S-transferase; TG – thyroid gland; p – reliability of index differences compared to control group; p_{AA} – reliability of index differences compared to the carriers of AA-genotype; p_{AG} – reliability of index differences compared to the carriers of AG-genotype.

gene with the oxidative stress OMP (F=123.0, p=0.008), the activity of the antioxidant defense system in GP and GST (F=123.0, p=0.01), as well as the markers of apoptosis with caspases-3 (F=123.0, p<0.001) and –8 (F=123.0, p=0.013) (Table 6).

There were more patients with thyroid pathology and increased degree of OMP and the activity of caspases –3 and –8 in thyroid tissue homogenate, against the background of significant AOP reduction (> 50 percentiles) than those with a moderate increase (≤50 percentiles) of OMP and caspases and a decrease in the activity of AOP by 3.60-3.08 times (p <0.001), respectively, without reliable differences between the genotypes of the *Fas* (*rs*2234767) gene (Table 7).

The significant increase of OMP (> 50 percentiles) and the activity of caspases –3, –8, together with the reduction of the activity of AOP (GP and GST), increase the risk of thyroid pathology by 2.96 and 7.5 times, regardless of polymorphic variants of the BCL-2 (rs17759659) gene. When the patient's

Table 7. Indices of oxidative status, antioxidant defense and the activity of caspases-3 and 8 in the thyroid tissue considering the polymorphic variants of the APO-1/Fas (rs2234767) gene.

Indices	Changes in indices, n	Genotypes of *Fas* gene, n=125 (%)	
		AG, n=23	GG, n=102
Degree of OMP	Moderate increase (≤50 percentiles), n=30	5 (21.74)	25 (24.51)
	Significant decrease (>50 percentiles), n=95	18 (78.26)	77 (75.49)
	χ^2; p	χ^2=14.70 p<0.001	χ^2=53.02 p<0.001
GP and GST activity	Moderate decrease (≤50 percentiles), n=30	5 (21.74)	25 (24.51)
	Significant decrease (>50 percentiles), n=95	18 (78.26)	77 (75.49)
	χ^2; p	χ^2=14.70 p<0.001	χ^2=53.02 p<0.001
Caspase-3, Caspase-8, un/ml	Moderate increase (≤50 percentiles), n=30	5 (21.74)	25 (24.51)
	Significant increase (>50 percentiles), n=95	18 (78.26)	77 (75.49)
	χ^2; p	χ^2=14.70 p<0.001	χ^2=53.02 p<0.001

Table 8. Polymorphic variants of the BCL-2 (rs17759659) gene as the risk factors of the thyroid pathology considering the apoptosis indices, protein oxidation and the antioxidant defense system

Genotypes of the *BCL-2* gene		*RelR*	*OR*	*95%CI RR*	*95%CI OR*	p
Increasing the degree of oxidative modification of proteins (>50 percentiles)	AA	7.5	66.0	2.54-22.12	6.03-722.02	<0.001
	AG,GG	2.96	8.79	2.13-4.13	4.85-15.95	<0.001
Decreasing the activity of glutathione peroxidase and glutathione-S-transferase (>50 percentiles)	AA	7.5	66.0	2.54-22.12	6.03-722.02	<0.001
	AG,GG	2.96	8.79	2.13-4.13	4.85-15.95	<0.001
Increasing the activity of caspases-3, 8 (>50 percentiles)	AA	7.5	66.0	2.54-22.12	6.03-722.02	<0.001
	AG,GG	2.96	8.79	2.13-4.13	4.85-15.95	<0.001

Note: *RelR* –relative risk; *OR* –Odds Ratio; *95%CI RR, OR* –confidence interval

Table 9. Polymorphic variants of the CTLA-4 (rs231775) gene as the risk factors of the thyroid pathology considering the apoptosis indices, protein oxidation and the antioxidant defense system.

Genotypes of the ***CTLA-4*gene**		*RelR*	*OR*	*95%CI RR*	*95%CI OR*	p
Increasing the degree of OMP (>50 percentiles)	AA	6.64	28.72	228-19.34	7.35-112.21	<0.001
	AG,GG	0.83	0.36	0.67-1.02	0.10-1.36	>0.05
Decreasing the activity of GP and GST (>50 percentiles)	AA	6.64	28.72	2.28-19.34	7.35-112.21	<0.001
	AG,GG	0.83	0.36	0.67-1.02	0.10-1.36	>0.05
Increasing the activity of caspases-3, 8 (>50 percentiles)	AA	6.64	28.72	2.28-19.34	7.35-112.21	<0.001
	AG,GG	0.83	0.36	0.67-1.02	0.10-1.36	>0.05

Note: RelR –relative risk; OR –Odds Ratio; 95%CI RR, OR –confidence interval.

Table 10. Polymorphic variants of the APO-1/Fas (rs2234767) gene as the risk factors of the thyroid pathology considering the indices of apoptosis, protein peroxidation and antioxidant defense system.

Genotypes of the ***APO-1/Fas* gene**		*RelR*	*OR*	*95%CI RR*	*95%CI OR*	p
Increasing the degree of oxidative modification of proteins (>50 percentiles)	AG	1.04	1.17	0.81-1.32	0.39-3.47	>0.05
	GG	0.96	0.85	0.76-1.23	0.29-2.54	>0.05
Decreasing the activity of glutathione peroxidase and glutathione-S-transferase (>50 percentiles)	AG	1.04	1.17	0.81-1.32	0.39-3.47	>0.05
	GG	0.96	0.85	0.76-1.23	0.29-2.54	>0.05
Increasing the activity of caspases-3, 8 (>50 percentiles)	AG	1.04	1.17	0.81-1.32	0.39-3.47	>0.05
	GG	0.96	0.85	0.76-1.23	0.29-2.54	>0.05

Note: *RelR* – relative risk; *OR* – Odds Ratio; *95%CI RR, OR* –confidence interval

genotype contains the wild allele A in the homozygous status, this risk is increased by 7.5 times than in the presence of the patient genotype A wild allele in the heterozygous state, 7.5 times higher than in the carriers of AG or GG genotypes (oR = 66,0; 95% CI oR = 6,03-722,02; p < 0.001 and OR = 8,79; 95% CI OR = 4,85-15,95; p <0.001), respectively (Table 8).

The analysis of the polymorphic variants of the *CTLA-4* (*rs231775*) gene showed that a significant increase in the degree of OMP and in the activity of caspases-3, –8, together with a low activity of AOD (GP and GST), become independent factors, increasing the risk of thyroid pathology (AIT and TA) by 6.64 times, but only when the patient has the main A-allele of the *CTLA-4* gene in the homozygous status (*AA*-genotype) (OR=28.72; 95%CI OR=7.35-112.21; p<0.001) in their genotype (Table 9).

Increasing OMP, decreasing the activity of AOP, as well as caspase-dependent mechanisms of apoptosis induction considering the polymorphic variants of the *APO-1/Fas* (*rs2234767*) gene, do not increase significantly the risk of AIT and TA in the population of the Northern Bukovina residents (Table 10).

Conclusions

1. The imbalance between the activity of peroxidation and antioxidant defense in patients with NGAIT and TA is associated with the promoter of the CTLA-4 and APO-1/Fas genes and is characterized by an increasing degree of OMP in the altered thyroid tissue, together with a lower capacity of the AOP system enzymes.

2. The activity of caspase-dependent apoptosis mechanisms is associated, to the greatest extent, with the promoter of the APO-1/Fas gene, almost 8 times weaker with the polymorphic site of the CTLA-4 gene and 13 times weaker with the polymorphic site of the BCL-2 gene: the activity of the effector and the initiator caspases-3 and –8 are higher than in control group.

3. Polymorphic variants of the CTLA-4 (rs231775) gene are associated with the processes of peroxidation and antioxidant activity of the initiator caspase-8: in patients with A-allele of CTLA-4 gene, the degree of OMP in the thyroid tissue is higher, with lower enzyme activity of AOP.

4. The high activity of OMP and caspases –3, –8, as well as the lower intensity of AOP, are independent factors that increase the risk of thyroid pathology (AIT and TA).

5. Increased OMP, reduced activity of AOP and caspase-dependent mechanisms of apoptosis induction, considering the polymorphic variants of the *APO-1 / Fas* gene, do not increase significantly the risk of AIT or TA in the population of the Northern Bukovina residents.

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