на тривале відведення сечі, або, іншими словами, безтрубчата деривація сечі (везикостома або уретерокутанеостома), про що ми повідомляли раніше [2, 3].

**Висновки**

Отже, порушення функції товстого кишечника повинно обов'язково супроводжуватись визначенням функції сечового міхура. При підтвердженні порушення накопичувально-евакуаторної функції сечового міхура, курс лікування повинен бути скерований на ефективну деривацію сечі, яка, у окремих випадках, може полягати у хірургічних методах відведення сечі (безтрубчаті методи) та комплексній ренопротекторній терапії при ознаках порушення функції нирок.

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**Значение констипационного синдрома в диагностике и лечении нервно-мышечной дисфункции мочевого пузыря у детей**

***Резюме.*** Установлено, что у детей существует тесная связь между констипацией и проблемами мочевых путей, включая инфекции, ночное недержание мочи (энурез), пузырно-мочеточниковый рефлюкс и расширение верхних мочевых путей [Averbeck M.A., Madersbacher H., 2011; Muhammad S. et al, 2015]. J Pannek et al. (2009) описали случаи задержки мочеиспускания вследствие массивной констипации, когда каловые массы перекрывали мочевые пути. Erhun Kasirga et al. (2006) установили значительно большую частоту инфекций мочевых путей у больных детей с констипационным синдромом. Veiga ML et al. (2013) установили, что дети с нейрогенной дисфункцией мочевого пузыря имеют большие шансы на запор, чем те, в которых отсутствуют симптомы нижних мочевых путей.

На основе полученных литературных данных и результатов собственных наблюдений, установлена взаимосвязь между констипационным синдромом и нервно-мышечной дисфункции мочевого пузыря у детей.

Таким образом, нарушение функции мочевого пузыря в контексте ее корреляции с констипационным синдромом, является актуальной проблемой, которая из-за своей многовекторности требует дальнейшего изучения и разработки как новых диагностических алгоритмов, так и комплексного лечения.

***Ключевые слова: констипационный синдром, нервно-мышечная дисфункция мочевого пузыря, дети.***

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**Meaning of Constipation Syndrome in the Diagnosis and Treatment of Neuromuscular Dysfunction of the Bladder in Children**

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***Abstract.*** Children were found to have close connection between constipation and urinary tract problems including infections, bedwetting (enuresis), vesicoureteral reflux, and the dilation of the upper urinary tract [Averbeck M.A., Madersbacher H., 2011; Muhammad S. et al, 2015]. J Pannek et al. (2009) described cases of urinary retention as a result of massive constipation when stool blocked urinary tract. Erhun Kasirga et al. (2006) found a significantly greater frequency of urinary tract infections in children of patients with constipation syndrome. Veiga ML et al. (2013) found that children with neurogenic bladder dysfunction have greater chances of constipation than those who have no lower urinary tract symptoms.

Based on the literature data and the results of our own observations, the interrelation between constipation syndrome and neuromuscular dysfunction of the bladder in children was established.

Thus, urinary bladder dysfunction in the context of its correlation with constipation syndrome is an urgent problem, which requires further study and development of a new diagnostic algorithms, and comprehensive treatment due to its multi-vector nature.

***Keywords: constipation syndrome; bladder neuromuscular dysfunction; children***.

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## Caspase-3 and Caspase-8 in Patients with Nodular Goiter and Autoimmune Thyroiditis

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***Abstract.*** The article presents results of the comparative analysis of peroxidation process activity of caspase-3 and caspase-8 in patients with thyroid adenoma (TA) and nodular goiter with autoimmune thyroiditis (NGAIT). Studying peroxidation processes in the tissue of the thyroid gland abnormal tissue was detected to be characterized by increase in of protein oxidative modification (POM) indices at the same time, the antioxidant enzymes activity (AEA) was significantly reduced and was more likely in patients with NGAIT. Significant increase in activity of both caspase 3 and 8 was shown in patients with NGAIT compared to macroscopically unchanged tissue and thyroid adenoma. Possible mechanisms of the detected disorders were discussed.

***Keywords: nodular goiter with autoimmune thyroiditis; thyroid adenoma; apoptosis; peroxidation; caspase 3; caspase 8.***

**Problem statement and analysis of the recent research**

Cells are exposed to many damaging factors of endogenous and exogenous nature in the process of their vital activity. Various toxic influences or metabolic disorders undoubtedly 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 [1-5]. One of cell responses in case of not only the toxic effects action, but also imbalance of growth factors, hormones, cytokines, when DNA is damaged, other structural elements of the cell or when the cell cycle course is interrupted, is activation of the genetic program of the cell death, that is apoptosis [6-15].

According to the literature, the earliest stage of apoptosis is oxidation of proteins and cell membranes, which is influenced by an excessive production of reactive oxygen species (ROS) [3,5]. The processes that allow the cell to adapt to the negative impacts and determine the possibility of its further existence or death, are believed to depend on the characteristics of the mechanisms of induction and regulation of apoptosis and its implementation [10].

Mechanisms of apoptotic death in autoimmune thyroid diseases have been a subject of an active study for the last decades [8, 10-14].

Intensity of studies on apoptosis in recent years has been connected with a number of circumstances. First of all, methodological possibility of registering various manifestations of apoptosis and analysis of its molecular mechanisms are closely related to mechanisms other current events (for example, activation of peroxidation) [1-4]. In addition, the study of apoptosis has been very productive for understanding a number of important processes, including immune homeostasis and oncogenesis [14]. Finally, due to apoptosis, it became necessary to reconsider a number of conceptual bases of pathophysiology [10].

Nowadays, a number of mechanisms of thyrocytes apoptosis induction have been found. Normally, apoptosis occurs in 2 major ways: internal way, mediated by mitochondria, resulting from activation of caspase 9, while the outer way is mediated by activation of the Fas-receptors (CD95) and includes the activation of caspase 8 [10,14]. Both ways converge to the activation of effectors' caspases, executors of apoptosis (caspase 3, 6, 7) leading to DNA degradation and cell death [15,16].

Proteins whose degradation causes irreversible changes in the cell are targeted by effectors caspase [10-12]. Caspases action is specific: under their influence only certain proteins degrade to fragments of a certain length [15]. Therefore, caspases make up a central component of apoptosis program, their activation leads to the final stage of the cell death, namely, to DNA fragmentation and degradation of structural proteins of the cytoskeleton and cell membranes as well as to inactivation of other proteins ensuring normal functioning of the cells [16]. The appearance of such protein fragments serves as a biochemical marker of apoptosis [14-16]. However, the literature does not give any serious data on the relationship of lipid peroxidation processes and activation of caspase cascade in patients with NGAIT.

**The objective** of the research was to study pro- and anti-oxidative activity, caspase-dependent mechanisms of apoptosis' induction in the thyroid tissue of patients suffering from autoimmune thyroiditis.

**Materials and methods**

75 women complaining of discomfort in the neck were examined during 2013-2015. We evaluated the hormonal status (with thyroid stimulating hormone (TSH), free T4 thyroxin, free triiodothyronine T3) levels of antibodies to thyroglobulin (AB-TG) and to thyroid peroxidase (AB-TPO), the volume and structure of the thyroid gland (TG) according to ultrasound examination.

25 women (group I) were diagnosed with thyroid adenoma after the surgery according to the ultrasound, fine-needle aspiration biopsy (FNAB) and histological findings. We identified this group due to the fact that this pathology is one of the most common forms of nodular goiter.

50 women were diagnosed with NGAIT (group II). The indications for the surgery in this group were: enlargement of the thyroid gland with symptoms of compression and narrowing of the trachea and esophagus; some nodes compressed organs of the neck; progressive growth of goiter, despite ongoing conservative therapy for 1.5 years; suspected malignant degeneration, based on FNAB findings.

The study did not involve the patients with hyperthyroidism, those manifesting hypothyroidism, hypertension and cardio-vascular diseases, as well as with severe somatic pathology and after the onset of menopause.

The patients of groups I and II were comparable in age (34.2±10.33 and 38.0 ± 10.62 years respectively, p = 0.12), anthropometric data (body mass index - BMI under 23.5 ± 2.71 and 24.3 ± 4.88 kg/m$^2$, p=0.43) and the level of free T3 (4.4 ± 0.91 and 4.4 ± 0.93 ng / L, p=0.93) but differed in terms of free T4 (16.6 ± 2.02 and 12.9±3.42 mmol / L, p <0.0001), TSH (1.9 ± 0.76 and 4.93 ± 51 mU / L, p<0.0001) and AT-TPO (11.9 ± 13.92 and 255.7 ± 340.58 mU / L, p=0.0009). In general, the differences between the groups were naturally determined and confirmed autoimmune destruction and tendency towards depression of the function on the background of NGAIT in patients from group II.

All the patients underwent surgery. Surgical interventions ranged from hemithyroidectomy to thyroidectomy. After the intervention the tissue was taken in the operating room no later than 30 minutes after the operation. In patients of group I we isolated separately macroscopically unchanged (paranodular) tissue, which served as the control one for both comparison groups, and adenomatous tissue. In patients with NGAIT 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 of 50-70 mg each. After the partition they were closed in a special plastic container and stored at -70 ° C before basic research with them.

In addition, we investigated pro- and antioxidant activity in 5% of thyroid tissue homogenates determining the activity of glutathione peroxidase (GP mmol / min • g tissue), Glutathione-S-transferase (GST, umol / min • g tissue) and degree of oxidative modification of proteins (OMP, optical density unit / g protein) by means of accepted methods.

In order to study the activity of caspases 3 and 8 thyroid tissue was crushed in homogenizer "WiseTis" HG-15 series ("Daihan Scientific", South Korea) with 8mm rotor at a speed of 4500 rev / min. For this purpose, the isolating medium (20 mM HEPES, pH 7.5, 10 mM KCl, 1.5 mM MgCl2, 1 mM DTT) was used 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 immunosorbent (ELISA) "Sanrise ™ -Tecan" (Austria), at a wavelength of 405 nm, with the speed of splitting the synthetic substrate N-Acetyl-Asp-Glu -Val-Asp-nitroanilin (Ac-DEVD-NHA) and N-Acetyl-Ili-Glu-Asp-Tre-nitroaniline (Al-IETD-PNA) respectively. All reagents used in this study were produced by the company "Sigma" (USA). Caspase activity was assessed in (mmol of paranitro-aniline/ [h • mg of protein]).

**Results of the research and their discussion**

The study of peroxidation processes in the thyroid tissue established that there was a significant increase in OMP parameters in the modified tissue at the same time, the activity of antioxidant enzymes (AOE) was significantly reduced, especially in patients with NGAIT (Fig. 1).

For instance, the activity of GP in patients of group I decreased by almost 15% compared to the paranodular tissue and by 18% in patients of group II. ST-T level decreased by 49.5% in patients of group I and by 56.8% in patients of group II. POM degree was 24% higher in patients of group I and by 33.4% in those of group II.

In the course of the study caspase-3 activity in the tissue having signs of NGAIT was found to be twice higher than in the unchanged thyroid tissue (Fig. 2) indicating the activation of caspase-dependent way of apoptosis under these conditions. In this case, the activity of caspase-8 increased significantly compared to both that of intact thyroid tissue and to patients of group I.

Such imbalance between the activity of peroxidation processes and antioxidant defense systems created the conditions for damaging action of peroxidation processes on the thyroid structures and for ROS impact on pro- and antiapoptic targets and mechanisms directly or indirectly through the intracellular redox-dependent signal-conveying systems. We consider these structures to be the elements of the thyrocytes, namely cell membrane, intracellular structures, which cause launching of

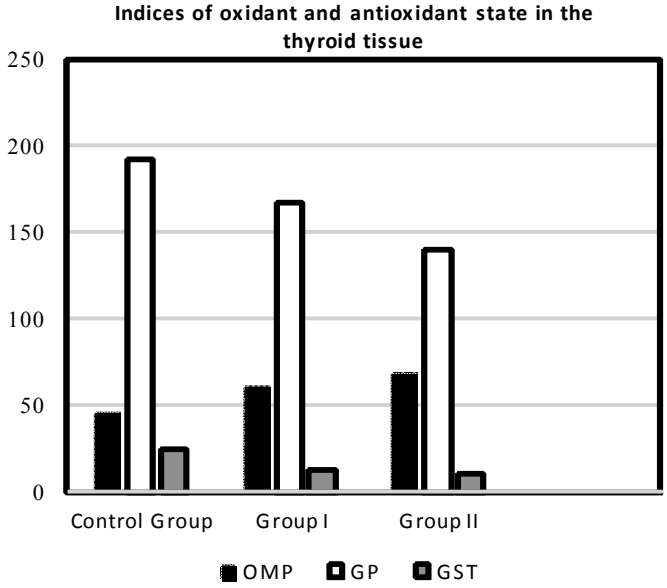


**Fig. 1.**

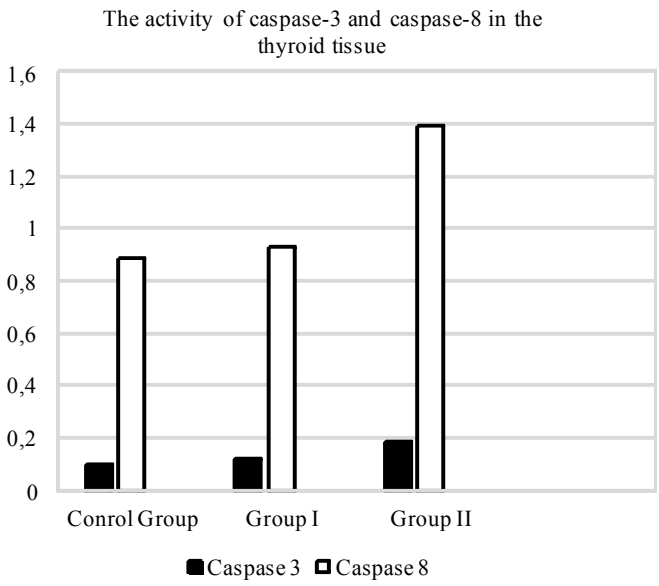


**Fig. 2.**

apoptotic signal, the indication of which is likely activation of both initiator and effectors caspases.

Additionally, several molecular paths interacting with each other can be activated in the cell. The findings of different sources confirm the role of antiapoptotic protein Bcl-2 under the condition of NGAIT development and this protein is among the main factors in the regulation of apoptotic function of mitochondria [10-13].

Oxidative stress leads to the formation of cell membranes of oxidated lipids which are also apoptotic factors. Caspase oxidative modification (including caspases-3), which are sensitive to cells redox status, depending on the type and location of such changes may cause their activation.

Considering this, as well as the findings obtained while performing the work we can assume that basic mechanisms of apoptosis are triggered in patients with NGAIT due to excessive activation of peroxidation aimed at attracting external receptor mechanism of initiation and increased activity of caspase-3, and can also occur as a result of caspase-8, the indicative of which is probable high increase in the activity of this index both as compared to that in unchanged tissue in the thyroid gland and in patients with thyroid adenoma.

The research of caspase signaling pathways in apoptosis of thyroid cells has recently started and requires further study. Discovery of physiological regulators of apoptosis in caspase activity shows the inexhaustible possibilities of cells to maintain homeostasis and the natural end of life cycle. Tracking ways causing cell death may contribute to the development of new approaches to the prevention and treatment of autoimmune thyroid disease.

**Conclusions**

1. Processes of protein peroxidation are activated and the systems of antioxidant defense become weaker in the thyroid tissue of patients with autoimmune thyroiditis (NGAIT).
2. Induction of thyrocytes apoptosis in patients with NGAIT on external mechanism is associated with increased activity of caspase-8, which significantly predominates in patients with thyroid adenoma and in practically healthy people by 56.18% and 49.46%, with an implementation through effectors caspase-3 the activity of which grew almost twice.

**Prospects for further research**

Detection of pathogenic factors and mechanisms of apoptosis deregulation in case of NGAIT will allow determining the additional causes of their onset and formulate pathogenetically grounded methods of immunopathological changes correction.

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