

ORIGINAL PAPER

REMOTE RESULTS OF HEMITHYROIDECTOMY AND THEIR PROGNOSTICATION IN PATIENTS WITH UNILATERAL NODULAR GOITER COMBINED WITH AUTOIMMUNE THYROIDITIS

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ABSTRACT

Background. As a result of the histological examination of the removed thyroid tissue, autoimmune thyroiditis has been diagnosed in 10.4% of patients. During the surgical treatment and while choosing the surgery volume, the pathogenetic mechanisms of the onset and progression of autoimmune processes in the thyroid tissue are not taken into account.

Materials and methods. The immunohistochemical study was carried out using monoclonal antibodies against Ki67, Fas, FasL, Bcl-2, and P53 anti-genes on a puncture material of the thyroid glands obtained from 80 patients with a histologically verified diagnosis of nodular goiter combined with autoimmune thyroiditis.

Results. The results of the study showed high proliferative activity of the lymphoid tissue, moderate proliferative activity of thyrocytes in the area of lymphoid

RÉSUMÉ

Résultats lointains de l'hémithyroïdectomie et leur pronostic chez les patients avec goitre nodulaire unilatéral combine avec thyroïdite auto-immune

Introduction. Comme résultats de l'examen histologique du tissu thyroïdien prélevé on a diagnostiqué une thyroïdite auto-immune chez 10,4% des patients. Pendant le traitement chirurgical et dans le choix du volume de l'opération les mécanismes pathogènes du début et de la progression des procès auto-immuns dans le tissu thyroïdien ne sont pas pris en considération.

Méthodes. En matière de ponction thyroïdienne obtenue à partir de 80 patients avec le diagnostic de goitre nodulaire sur le fond de la thyroïdite auto-immune vérifié du point de vue histologique, on a effectué des

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infiltration which was low outside of it and which was taken into account when determining the volume of surgical intervention.

Conclusions. The results of the study have shown that thyroidectomy is a pathogenetically grounded operation in the case of unilateral nodular goiter combined with autoimmune thyroiditis with pronounced proliferative activity of the thyroid tissue in contralateral lobe.

Key words: nodular goiter combined with autoimmune thyroiditis, needle biopsy, apoptosis, proliferation, volume of surgery.

Abbreviations: NGAIT – nodular goiter with autoimmune thyroiditis, AIT- autoimmune thyroiditis, TG – thyroid gland, PCD – preoperative cytological diagnosis.

INTRODUCTION

The problem of autoimmune thyroiditis (AIT) is one of the pressing clinical problems, the solution of which requires further integrated research. It is determined by the fact that AIT is the most common thyroid disease and involves 46% of all thyroid pathologies^{1,2}.

Its incidence constitutes from 6 to 10% among adult population and from 0.1 to 1.2% among children^{1,3}. AIT incidence does not tend to decrease and has increased by 10 and more times for the last 6-10 years^{1,4}.

Nodule formation against the background of AIT is found by histological examination in 1/3 of patients^{3,7}. Surgeons face the problem of operations on the thyroid gland in hyperdiagnosis of nodule formation against the background of AIT. The simplified approach, which is only based on ultrasound findings, leads inevitably to unreasonable operations the frequency of which reaches 21%^{6,8}. At the same time, the diagnosis of a true nodule formation against the background of AIT has certain difficulties, which are associated with specific changes in the thyroid gland in this disease⁹⁻¹¹.

Despite of using a complex of modern diagnostic methods, it is not always possible to establish the morphological nature of nodular structures in the

études immunohistochimiques en utilisant des anticorps monoclonaux contre le Ki-67, Fas, FasL, Bcl-2 et les antigènes p53.

Résultats. Les résultats ont montré une forte activité proliférative du tissu lymphoïde, l'activité proliférative modérée dans la zone des thyrocytes dans la région de l'infiltration lymphoïde et faible en dehors, ce qui a été pris en compte lors de la détermination du volume de la chirurgie.

Conclusions. Les résultats de l'étude ont montré que la thyroïdectomie est une opération motivée du point de vue pathogénique en cas de goitre nodulaire unilatéral sur le fond de thyroïdite auto-immune avec une activité proliférative sévère du tissu thyroïdien dans le lobe controlatéral.

Mots-clés: goitre nodulaire sur le fond de la thyroïdite auto-immune, biopsie à l'aiguille fine, apoptose, prolifération, volume de l'opération.

Abbréviations: NGAIT- goitre nodulaire sur le fond de la thyroïdite auto-immune, AIT- thyroïdite auto-immune, TG- glande thyroïde, PCD- diagnostic cytologique pré-opérationnel

thyroid gland against the background of AIT correctly.

Unfortunately, the chemical reagents used in preparation of specimens to morphological studies by the standard method block most of the antigenic determinants. Therefore, the immunocytochemical and morphological studies of puncture material are carried out on separate specimens, which leads to additional puncture biopsies and prevents the morphological identification of the cells reacting with antibodies. Instead, the optimum for preoperative cytological diagnosis PCD is the option where the cytomorphological and immunocytochemical study is performed sequentially on the same smear of puncture material¹²⁻¹³.

A disorder in the regulation of the cell cycle with inhibition of apoptosis and activation of proliferation is one of the mechanisms of tumor transformation and progression¹⁴⁻¹⁹.

At present the markers of the apoptosis regulation tend to include the membrane receptors Fas and Fas-L, proteins Bcl-2; with some researchers pointing out the key role of proto-oncogene Bcl-2 in apoptosis regulation¹⁴⁻¹⁵. In addition to proteins of the Bcl-2 family, a gene suppressor nuclear phosphoprotein p53 is the apoptosis regulator as well^{14,17,18}. This gene occurs in two types: the p53 wild type is thought to stimulate apoptosis, while the mutant p53 has a similar effect

on apoptosis as the Bcl-2 protein that suppresses programmed cell death. There are about 500 mutations of this gene¹⁸. Mutations of this protein in patients with malignant, benign and autoimmune thyroid diseases have been insufficiently studied^{11,18}.

In addition to the factors of apoptosis, proliferative activity presents a very important information for determining the essence of the pretumor state and its prognosis in terms of malignancy. Ki-67 antigen is one of the immunohistochemical markers of proliferation. Ki-67 proved to be present on the cell nuclei in all stages of the life cycle, except G0 and G1 in the initial stage. After the cell leaves the mitotic cycle, the antigen cannot be detected^{14,17,19,20}. Expression of these molecules can be detected by standard immunocytochemical methods, which are easy to integrate into the process of fine-needle aspiration biopsy (FNAB). Using additional diagnostic techniques, such as immunohistochemistry, can help in the interpretation of „unnoticed“ changes, but at present there are no conventional standard molecular markers^{10,11,13,15}.

It is absolutely necessary to solve these problems, because the right choice of treatment tactics, the timeliness of surgical treatment, and, as a result, the survival of the patient depends largely on the accuracy of PCD^{11,12}.

OBJECTIVE

To determine the volume of surgical intervention for nodular goiter combined with autoimmune thyroiditis, taking into account the activity of apoptosis and proliferation processes and the proliferative activity index.

MATERIALS AND METHODS

The study involved 80 women with NGAIT treated in the Chernivtsi Regional endocrine specialized clinic. The age of patients ranged from 23 to 72 years. The diagnosis was made clinically, in laboratory – thyroid peroxidase antibody (TPOab) – 60-250 units/mL; thyroglobulin antibody (TGab)– 60-500 units/mL; thyroid stimulating hormone (TSH) – 4-10 mUn/L; free thyroxine (fT4) – 7.7-14.2 pmol/L with ultrasound and confirmed histologically after the surgical treatment.

All patients underwent surgical intervention according to generally accepted indications: large size of the goiter with compression and displacement of the neck organs (compression syndrome), airway obstruction or suspected malignant neoplasm of the thyroid gland (III, IV, V classification groups of The Bethesda system for reporting thyroid cytopathology) according

to FNAB (fine needle aspiration biopsy). The volume of the surgery is hemithyroidectomy.

The study did not include patients with hyperthyroidism, overt hypothyroidism, arterial hypertension and cardiovascular diseases, severe somatic pathology and those after the menopause onset.

All patients underwent ultrasound-controlled FNAB of nodes, outnodular parenchyma and the contralateral thyroid lobe, with at least 3 punctures, based on the fact that the percentage of inadequate punctures decreases, depending on their number in the following proportion: 1 puncture – 16%, 2 punctures – 5.3%, 3 punctures – 4%, 4 punctures – 2.6%¹⁰.

In the preparation of the smears, a way to restore the activity of antigenic determinants developed and patented in the laboratory of the Institute of Endocrinology named after V.I. Komisarenko was used. It allows combining cytomorphological and immunocytochemical studies on one cytological specimen and enables comparing the morphological and immunocytochemical characteristics of individual cellular elements⁹.

This method provides reliable results on the specimens that were stored for no more than three days after staining. After this period, the results are unstable, which is due to the processes of oxidation of some chemical compounds exposed to the air^{9,12,13}. To model an immunohistochemical reaction, monoclonal antibodies against the following antigens were used: Mouse Human Ki-67 FITC Clone MIB-1; Anti-p53 Protein Monoclonal Antibody, FITC Conjugated, Clone DO-7; Mouse Anti-Human Apoptosis Regulator Bcl-2 (BCL2) Monoclonal, Unconjugated, Clone 124 antibody; Mouse Anti-Human CD95 Monoclonal Antibody, Unconjugated, Clone FAS 18; Mouse Anti-Human CD95L Monoclonal Antibody, Unconjugated, Clone NOK-1 by Dako Denmark A / S (Denmark).

The results of the immunohistochemical reaction were evaluated by the semi-quantitative analysis proposed by O. K. Khmel'nitsky, according to the intensity of staining „+“ – insignificant, „+“ – weak, „++“ – moderate, „+++“ – pronounced¹³. The evaluation of immunoreactive cells was calculated using the formula IRI (immunoreactive index) (Fas, FasL, Bcl-2, P53) = $N1/N2 \times 100\%$, where N1 is the number of cells immunopositive to Fas, FasL, Bcl-2 and P53 receptors, N2 is the total number of cell nuclei per 1 square millimeter. The evaluation of the PAI (proliferative activity index) was based on the formula PAI = $NKi67 / N \text{ unit} \times 100\%$, where NKi67 is the total number of nuclei immunopositive to Ki67 protein, N units. – total number of nuclei of cells per 1 mm². The morphometric analysis was carried out by means of a microscope Bresser BioScience Bino 40x-1000x

(Germany) with a digital camera Nikon DS-fil, a personal computer with the software NIS-Elements F 3.2.

RESULTS

The immunohistochemical study found that in all cases (100%) Ki67 expression on the thyrocytes was weak „+“ and moderate „++“, which manifested itself in the form of brown or light brown staining of the thyrocytes nuclei in the experimental and control group patients. The number of immunoreactive cells in the specimen was distributed unevenly. There was an increased expression of Ki67 and PAI varied from 9.75 to 11.37% (median 10.56%) in the thyrocytes, near the centers of destruction of the thyroid epithelium, near the foci of lymphoid infiltration. When counting the number of immunoreactive thyrocytes that were out of lymphoid infiltration, there was a decrease in the index of proliferation from 8.75 to 11.37% (median 10,56%).

A significant number of the thyrocytes expressing Fas was detected. The cytoplasmic membrane of the thyrocytes was characterized by brown „++“ and dark-brown „+++“ staining. The number of immunoreactive cells varied from 47 to 63% (median 55%). It is noteworthy that significant expression of „+++“ was observed in places with pronounced lymphoid infiltration. Expression of FasL was higher in „+++“ in the follicles and adjacent regions of lymphoid infiltration „++“, which in the ratio of cells was on average 46.10%.

Bcl-2 was slightly „+“ expressed by the thyroid epithelium, in contrast to the foci of lymphoid infiltration, whose cytoplasm was stained brown „+++“. In the formation of foci of lymphoid infiltration without clear boundaries this tissue in the thyroid gland was stained evenly in the center and on the periphery of the follicles. When mature follicles were formed, there was a lack of staining in the center and an intense coloration of the mantle zone of the lymphoid follicle. The lymphatic infiltration of tissue outside the nodes was intensively expressed by Bcl-2 in the area of thyroid follicles and in the stroma of the gland. When analyzing the number of cells expressing Bcl-2, from 87 to 93% of immunoreactive cells were found (median 90%).

Our study found a high expression of protein p53 in the nuclei of thyrocytes and in the follicular lumens with a pronounced positive reaction and stained dark-brown nuclei. The color on this marker was of a local nature, and there were no more than 7-9 nuclei in one field of vision. The concentration of immunoreactive cells ranged from 64 to 71% (median 65.5%).

DISCUSSION

According to the literature, Ki-67 is one of the most commonly used immunohistochemical markers in the differential diagnosis between benign and malignant tumors in human pathology^{13,15}. As a result of our study, the index of proliferative activity was found to be the largest in the thyroid tissue with focal lymphoid infiltration and the lowest in the thyrocytes in patients of both groups. The conducted study suggests that patients with GNAIT had a suppression of apoptosis processes in the thyroid tissue, as evidenced by a reduction in the expression of Fas and FasL on the thyrocytes compared to the lymphoid infiltration regions. The co-expression of Fas and FasL in the lymphoid infiltration regions around the thyroid cells suggests that Fas and FasL are not directly involved in apoptosis of the thyroid cells, but induce this process by producing proapoptotic cytokines. This conclusion is confirmed by an insignificant expression of Bcl-2 by the thyroid epithelium, in contrast to the tissue with lymphoid infiltration, the cytoplasm of which was stained brown „+++“. According to the literature, the modification of protein expression of p53 in the thyroid gland more often correlates with the degree of differentiation of carcinoma of the gland. In poorly differentiated carcinomas, the expression of the protein p53 can be found between 40% and 62%, whereas in well-differentiated carcinomas it does not exceed 25%^{14,20}. The increased density of p53-positive cells can be explained by mutations in the p53 gene, which allows cells to find tolerance for the apoptotic effect of the immune system's effectors^{14,15,18,20}.

According to remote results of surgical treatment of unilateral nodular goiter combined with autoimmune thyroiditis (the observation period from 1.5 to 3 years), two groups of examined patients were selected. The patients of the 1st group were 53 individuals without any disorders in the functional state of the thyroid gland and, according to the ultrasound examination, the size of the remained thyroid lobe did not differ from the preoperative ones.

The second group consisted of 27 patients who, after the hemithyroidectomy, were re-examined and according to the ultrasound scan, had an increased remained thyroid lobe and the formation of the node or nodes in it against the background of severe lymphoid infiltration, and the levels of free thyroxine (fT4) and TSH indicated moderate hypothyroidism (Table 1).

Immunohistochemical studies of the punctures of the remained lobe in this group of patients found a reliable inhibition of apoptotic processes against the background of a pronounced activation of proliferative processes (Table 2). It should be noted that

Table 1. Comparative estimation of the lobe size and the functional state of the thyroid gland in patients after a hemithyroidectomy for unilateral nodular goiter combined with autoimmune thyroiditis

Values	Group I (n=53)		Group II (n=27)	
	Before operation	After operation	Before operation	After operation
the volume of the thyroid gland undamaged by the node (cm ³)	10.18 ± 0.25	9.27±0.23	10.42±0.91	20.86±1.17*
fT4 (pmol/L)	15.52 ± 0.19	13.8±0.19	13.02±0.21*	5.83±0.46*
TSH (mUn/L)	2.12 ± 0.14	2.37±0.15	3.08±0.45*	13.76±0.89*
TPOab (UN/mL)	73.21 ± 0.19	74.45±0.56	82.44±1.15*	215.68±1.88*
TGab (UN/mL)	73.15 ± 0.49	78.49±0.49	93.23±1.23*	135.27±1.52*

Note: * - P<0,05 - reliability index between the groups.

Table 2. Comparative assessment of the proliferation and apoptosis markers in patients after hemithyroidectomy for unilateral nodular goiter combined with autoimmune thyroiditis

Values	Group I (n=53)		Group II (n=27)	
	Before operation	After operation	Before operation	After operation
PAI Ki-67 (%)	8.45±0.22	8.85±0.23	10.91±0.24*	11.22±0,19*
IRI Fas (%)	51.21±0.17	55.63±0.27	41.16±0.54*	39.93±1.63*
IRI FasL (%)	41.71±0.19	45.63±0.19	56.17±0.76*	64.26±0.98*
IRI Bcl-2 (%)	86.71±0.18	90.64±0.17	98.33±0.85*	97.41±1.86*
IRI p53 (%)	66.13±0.15	65.19±0.15	54.72±0.61*	48.14±1.19*

Note: * - P<0,05 - reliability index between the groups.

patients of both groups received an adequate, pathogenetically substantiated conservative therapy in the postoperative period.

A retrospective comparison of ultrasound findings, those of hormonal capacity of the thyroid gland and of the antibodies titer of TPO and ATG established that in patients of the 1st and 2nd groups the volume of the lobe of the gland which had not been affected by nodes did not differ reliably before the surgery, whereas the difference in the rates of the thyroid status and AbPO and AbTG was reliable (Table 1).

When comparing the results of proliferation and apoptosis, it was found that the PAI Ki-67, IRI FasL and IRI Bcl-2 were higher while IRI Fas and IRI p53 were lower in the first group than those in the second one (Table 2).

It is evident that a reliable increase in the rates of proliferation and inhibition of apoptosis revealed in the distant period after the surgical treatment of NGAIT leads to functional insufficiency and hyperplasia of the contralateral lobe of the thyroid gland in patients with nodular goiter combined with autoimmune thyroiditis, which was found in patients of the 2nd group. In this regard, there is a need to review the choice of the surgery volume in patients

with NGAIT, depending on the severity of the autoimmune process and the functional capacity of the gland.

Therefore, the retrospective analysis allows us to delimit the choice of the operation volume - hemithyroidectomy, thyroidectomy, depending on the functional capacity of the thyroid gland and the rates of apoptosis and proliferation. When combining the studied parameters: the volume of the thyroid gland part unaffected by the node is more than 10 cm³, the TSH level is more than 3.55 mUn/L, of fT₄ is less than 12.91 pmol/L, the TPOab level is more than 80.25 UN/mL and the TGab level is more than 89.34 UN/mL with indicators of lymphoproliferative activity and apoptosis, in particular the levels of PAI Ki-67 higher than 10.55%, IRI FasL than 51%, IRI Bcl-2 than 90%, and reduced values of IRI p53 lower than 50%, and IRI Fas than 43%, thyroidectomy should be preferred.

CONCLUSIONS

1. In case of unilateral nodular goiter combined with autoimmune thyroiditis and elevated proliferation in the tissue of the node and the low one in the

contralateral lobe, hemithyroidectomy in combination with a developed conservative therapy complex in the postoperative period is the operation of choice.

2. In patients with unilateral nodular goiter combined with autoimmune thyroiditis and pronounced proliferative activity in the tissue of the contralateral lobe, thyroidectomy is the pathogenetically substantiated operation.

3. The volume of gland unaffected by the node more than 10 cm³, the TTH level more than 3.55 mU_n/L, fT₄ less than 12.91 pmol/L, TPOab rate more than 80.25 UN/mL, TGab more than 89,34 UN/mL, PAI Ki-67 more than 10.55%, IRI FasL elevated by more than 51%, IRI Bcl-2 is higher by 90%, IRI Fas is lower by 43% and IRI p53 lower by 50% can be considered to be a risk factor of functional insufficiency and hyperplasia of the contralateral lobe of the thyroid gland after hemithyroidectomy in patients with nodular goiter combined with autoimmune thyroiditis.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

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