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FINE NEEDLE BIOPSY IN DIFFERENTIAL DIAGNOSIS OF NODULAR GOITER WITH AUTOIMMUNE THYROIDITIS AND DIFFERENTIATED THYROID CANCERS

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Abstract

We examined 75 women with nodular NGAIT and 12 patients with differentiated thyroid cancer during 2015-2016. We have carried out an immunohistochemical study by means of monoclonal antibodies against Fas, FasL, Bcl-2, P53 and Ki67 antigens using the thyroid gland puncture material.

Thus, compared with known methods of differential diagnosis, the proposed study allows for preoperative stage, with a high probability differentiate NGAIT of DTC, by examining extracts from tissues of the thyroid gland, which, in turn, said the volume of surgery and treatment program.

Abbreviations: NGAIT - nodular goiter combined with autoimmune thyroiditis, TG – thyroid gland, PCE – preoperative cytological examination, DTC- differentiated thyroid cancer.

Key words: nodular goiter with autoimmune thyroiditis, fine needle biopsy, apoptosis, proliferation, thyroid gland, differentiated thyroid cancer.

Introduction

One of the mechanisms of malignant transformation and progression is a cell cycle dysregulation with apoptosis inhibition and proliferation activation [1].

During embryonal development and morphogenesis, apoptosis may be induced by two pathways. The first is an external protein signal originating from other cell - also named as "death signal". The another one is a specific cell reaction to external stress factors. Apoptosis is regarded as one of the key processes in carcinogenesis and tumor growth. Apoptosis may be induced via cytokines (FasL) or protein p53 [2, 3].

FasL is a glycoprotein of cell membrane. It induces apoptosis in cells, which express on their surface Fas receptors. Quantitative and qualitative disorders in expression of these proteins may enable carcinogenesis [1 - 4].

In modern literature there are many publications dealing with a study of nodular goiter combined with autoimmune thyroiditis (NGAIT) morphology [1 - 5]. However, some issues remain unresolved including that about the role of autoimmune thyroiditis (AIT) in the development of tumor processes. According to the literature, AIT leads to metaplasia processes in the thyroid epithelium, hyperplasia of lymphoid tissue, which undoubtedly can be considered as an optional precancerous condition [4 - 8].

The information that papillary cancer and lymphomas occur three times more frequently in patients with NGAIT confirms this idea [3, 5 - 8].

The total accuracy of clinical, instrumental and laboratory diagnostic methods for the establishment of morphological origin of nodular new growths in the TG even in the most daring conclusions does not exceed 80% [1 - 8]. This result cannot be satisfactory either for surgeons (unjustified over diagnosis of thyroid cancer) or for endocrinologists (inadequate and ill-timed selection of patients for surgical treatment) [10, 11].

Unfortunately, the chemical reagents used in the preparation of drugs for morphological studies by a standard method, block most of the antigenic determinants. That is why immunocytochemical and morphological studies of the biopsy material is performed on individual drugs, which leads to additional needle biopsies and prevents from the

morphological identification of the cells reacting with antibodies. Instead, the best for PCE (preoperative cytological examination) is the option when cytomorphological and immunocytochemical study is carried out consistently on the same smear of a puncture material [12, 13].

One of the mechanisms of malignant transformation and progression is a cell cycle dysregulation with apoptosis inhibition and proliferation activation [14 - 20].

It is quite necessary to solve these problems, because the correct choice of treatment strategy, timely surgical treatment and therefore the patient's survival depend largely on the accuracy of PCE [11, 12].

That is why our aim was to study the processes of proliferation and apoptosis in thyroid puncture material under NGAIT using immunohistochemical method of investigation as well as determining the proliferative activity index.

Material and methods

We examined 75 women with nodular NGAIT and 12 patients with differentiated thyroid cancer during 2015-2016.

While preparing the smears we used a method of restoration of antigen determinants activity developed and patented in the laboratory of the V. I. Komisarenko endocrinology Institute. It enables to combine cytomorphological and immunocytochemical researches in one cytological preparation and provides a possibility to compare morphological and immunocytochemical characteristics of certain cellular elements [9].

This method gives good results on the drugs that were kept after staining no more than three days. After this period, the results were unstable, which is due to the oxidization processes in some chemical compounds in the air [9,12,13]. To start an immunohistochemical reaction we used monoclonal antibodies to the following antigens: 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 FAS18; Mouse Anti-Human CD95L Monoclonal Antibody, Unconjugated, Clone NOK-1 by Dako Denmark A/S.(Denmark)

The results of immunohistochemical reaction were evaluated by means of semiquantitative analysis, proposed by A.K. Khmelnytsky, according to the intensity of color "+ -" - small "+" - poor, "++" - moderate, "+++" - pronounced [13]. Assessment of immunoreactive cells was calculated by the formula (Fas, FasL, Bcl-2, P53, Ki-67) = N1 / N2 × 100%, where N1 is the number of immuno-positive cells to Fas, FasL, Bcl-2, P53, Ki-67

receptors, N2 - the total number of the cellular nuclei per 1 square millimeter. Morphometric analysis was performed by means of the microscope Bresser BioScience Bino (Germany) with a digital camera Nikon DS-Fil, personal computer with installed software NIS-Elements F 3.2.

Results and discussion

The results showed the degree of proliferative activity in the thyroid tissue NGAIT. A high proliferative activity of lymphoid tissue, moderate proliferative activity in the area of thyrocytes lymphoid infiltration and low - outside. Marked expression of Fas and FasL in t thyrocytes in areas of lymphoid infiltration indirectly indicates that when there NGAIT immunologically caused apoptosis thyrocytes. This has been an increase in the expression of FasL in patients punctate DTC, whereby a significantly ($P < 0.01$) in malignant tissue. This process can be explained FasL probability of participation in the program "death signal" to p53, which is part of the pathogenesis of activating apoptosis in response to any external stress.

Increasing the number of immunoreactive cells expressing Ki67 in the area of lymphoid infiltration and destruction thyrocytes, evidence of follicular epithelial regeneration - as a compensatory-adaptive response body. In the study differentiated cancers DTC found a high frequency of expression of Ki-67 in the follicular (100%) and papillary (78.95%) thyroid cancer. The highest expression of the power of a marker characteristic of follicular thyroid cancer first.

Severe Bcl-2 expression in lymphocytes prevents the entry of cells into apoptosis and prolongs cell survival. There was high expression of p53 protein in the nuclei and follicular lumens of the thyrocytes, can be explained by mutations in the gene p53, which allows cells to find tolerance apoptotic action of effector immune system. In tumor tissue DTC noted the greatest increase expression levels of p53 and Ki-67 in paranodular cells compared with benign tissue and altered in patients NGAIT.

Conclusions

Thus, compared with known methods of differential diagnosis, the proposed study allows for preoperative stage, with a high probability differentiate NGAIT of DTC, by examining extracts from tissues of the thyroid gland, which, in turn, said the volume of surgery and treatment program.

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