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Original scientific paper

KI67 EXPRESSION IN PAPILLARY THYROID CANCER

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ABSTRACT

Background. Papillary thyroid cancer is the most common thyroid cancer and accounts for over 70% of malignant thyroid diseases. In the last few decades the incidence of the papillary thyroid cancer (PTC) has increased worldwide. The aim of the study is to evaluate the Ki67 protein expression in papillary thyroid carcinoma and to correlate with clinicopathological parameters (age, gender, tumor size, vascular invasion, capsule invasion, lymph node metastasis, multifocality).

Materials and methods. The study population consists of 47 patients diagnosed with PTC, and benign thyroid changes. After standard surgical procedure formalin fixed paraffin embedded (FFPE) tissue sections for standard histological and immunohistochemically Ki67 analysis were obtained.

Results. Out of 47 patients, 24 (51.06%) were diagnosed with papillary thyroid cancer (Ki67expression varies in the range 8.58-5.76%) and 23 (48.94%) were benign changes of the thyroid gland (expression of Ki67 varies in the range 1.61±1.23%). For Z = 5.49 and p <0.001 (p = 0.000) the cancers had significantly higher Ki67 expression than benign tumors. There was no significant variation in Ki67 expression in papillary thyroid carcinoma in comparison to cervical lymph node metastases and tumor position. These parameters vary with tumor size and multifocality.

Conclusion. Ki67 is an appropriate biomarker used to distinguish papillary carcinoma from benign thyroid lesions. Ki67 expression was associated with tumor size and multifocality. High expression of Ki67 could be an important indicator for assessing the clinical course and prognosis of the disease itself.

Key words. Papillary thyroid cancer, expression and Ki67

BACKGROUND

Thyroid cancers are the most common tumors of the endocrine system and make up about 95% of all tumors of endocrine origin [1]. The development of thyroid cancers, similar to other cancers, uncontrolled cell proliferation must first occur. A number of hormones, growth factors, and steroids regulate the proliferation and function of normal and neoplastic thyroid tissue [2-4].

Cell proliferative activity is an important factor in evaluating the behavior of malignant cells, and Ki67 is one of the most commonly used markers for assessing the proliferative capacity of malignant cells. Ki67 protein expression is regulated by proteolytic processes, including mechanisms controlled by key regulatory complexes: cyclin B / cyclin-dependent kinases [5]. It is structurally similar to other proteins involved in cell cycle regulation [6].Ki67 is used in the evaluation of clinical progression and prognosis of malignant tumors. Relevant studies have reported strong protein expression of Ki67 in highly malignant tumors [7]. The prognostic value of Ki67 has been investigated in a number of studies in breast, thyroid, lung, prostate and central nervous system cancers [12-15].

The prevalence of papillary thyroid cancer is almost four times higher in women than in men [8,9] and decreases after menopause [10]. Papillary thyroid cancer is known to have a better prognosis than other malignant tumors of the human body, although about 10% show a worse clinical course than expected. Higher prevalence among women, especially during the reproductive period, is observed in all regions and in all ethnic groups [11].

MATERIALS AND METHODS

This study was performed at the University Clinic for Thoracic and Vascular Surgery at the University "St. Cyril and Methodius" in Skopje, Republic of Macedonia, while immunohistochemically and molecular analyzes of surgical specimens were analyzed at the Institute of Pathology, Faculty of Medicine, Skopje.

The study population consists of 47 patients that underwent thyroidectomy for PTC or subtotal thyroidectomy for benign thyroid disease (thyroid adenomas, goiter). Patients were separated in two groups: group A consists of 24 patients diagnosed with papillary thyroid cancer and group B consists of 23 patients (control group), patients with benign thyroid disease.

Preoperative examinations such as thyroid ultrasound, fine-needle biopsy, and gland scan are performed at the Institute of Nuclear Medicine and Pathological Physiology. Patients with papillary carcinoma are examined by CT scan of the neck with intravenous contrast at the Radiology Clinic, while laboratory tests are performed at the Institute of Clinical Biochemistry.

Surgical technique: Total thyroidectomy is performed under general anesthesia and endotracheal intubation in all cases. An incision of 4 to 6 cm is made in the lower parts of the neck. At that point the subcutaneous tissue and platysma are surgically dissected and we reach to a group of infrahyoid musculature that is dissected. As a result, we reach the thyroid gland, which is completely removed (for subtotal thyroidectomy we remove only the part of thyroid tissue that is pathologically altered) and we are careful to preserve the parathyroid glands and the n.laryngeus recurrens. When we have enlarged lymph nodes we continue with elective cervical dissection.

After gross dissection, formalin fixed paraffin-embedded (FFPE), 4 microns thick tissues sections were stained in standard protocol and used to determine: tumor location, tumor focality, size of the primary tumor, the presence of lymphatic or vascular invasion, extrathyroidal extension into perithyroidal soft tissue, number of lymph nodes with metastases, margin status and the stage of the disease. The stage of the disease was determined according to the criteria of the Union for International Cancer Control (UICC), 8th edition [19]. The Ki67 immunostaining was performed using DAKO monoclonal antibody (clone Mib1, dilution 1:150), by semi-automated PT Link immunoperoxidase technique. After deparaffinization and rehydration, samples were pretreated with Target Retrieval Solution for 20 minutes at 97°C and then incubated with primary antibody for 20 minutes at 25°C. For antibody detection EnVision FLEX, DAKO visualization system (20 minutes at 25°C) and chromogen -di–amino-benzene-DAB (5 minutes at 25°C) were used. At the end slides were counterstained with hematoxylin.

RESULTS

1. Gender

The study included 47 (100.0%) patients, of which 40 (85.1%) were women and 7 (14.9%) men (Table 1). Out of 40 (85.1%) women, 22 (46.8%) had benign changes in the thyroid gland and 18 (38.3%) were diagnosed with papillary thyroid cancer. Of 7 (14.9%) men, 1 (2.1%) had a benign finding and 6 (12.8%) were diagnosed with papillary thyroid cancer.

There was no significant difference in the cross-tabulation performed between the patient sex and the Fisher's Exact Test diagnostic finding p> 0.05 (p = 0.097) / Monte Carlo Sig, (2-sided).

Table 1. Gender / Crosstabulation

Benign Carcinoma			Type		Total
Gander	Female	Count	22	18	40
		%	46,8%	38,3%	85,1%
	Male	Count	1	6	7
		%	2,1%	12,8%	14,9%
Total		Count	23	24	47
		%	48,9%	51,1%	100,0%

1.1 Gender & Expression

In women, the expression value of Ki67 varies in the range 5.13-5.76% and in men the expression value of Ki67 varies in the range 5.43±3.46%. For Z = 00.96 and p> 0.05 (p = 0.34) men have a slightly higher expression of Ki67 than women (Table 1.1).

Table 1.1 Gender / Difference in expression

Variable	Rank Sum Female	Rank Sum Male	U	Z	p-level	Valid N Female	Valid N Male
Expression	928,00	200,00	108,00	-0,96	0,34	40	7

2. Age of patients

The age of the patients varies in the range 46.30 ± 12.48 years.

Out of a total of 47 (100.00%) patients, 22 (46.81%) were <45 years old (expression of Ki67 varies in the range 6.05-4.92%) and 25 (53.19%) had ≥ 45 years (expression of Ki67 varies in the range 4.40-5.97%). For Z = -1.63 and p> 0.05 (p = 0.10) patients who were <45 years of age had a slightly higher expression of Ki67 than patients who were ≥45 years of age (Table 2).

Table 2. Patient age / Ki67 expression

Variable	Rank Sum ≥ 45 yrs	Rank Sum < from 45 yrs	U	Z	p-level	Valid N	Valid N
Expression	523,50	604,50	198,50	-1,63	0,10	25	22

3. Tumor size

Tumor size varies in the range of 1.95-1.13 centimeters. Out of a total of 24 (51.06%) patients, 14 (29.79%) had tumor size <2 cm (expression of Ki67 varies in the range 7.43, 4.50%) and 10 (21.27%) had a tumor size ≥ 2 cm (expression of Ki67 varies in the range of 10.20 ± 7.10%). For Z = 00.88 and p> 0.05 (p = 0.38) patients who had a tumor size ≥ 2 cm had a higher expression of Ki67 than patients who had a tumor size <2 cm (Table 3).

Table 3. Tumor size / Ki67 expression

Variable	Rank Sum < from 2 cm	Rank Sum ≥ from 2 cm	U	Z	p-level	Valid N < from 2 cm	Valid N ≥ from 2 cm
Expression	160,000	140,000	55,00	-0,88	0,38	14	10

4. Capsule invasion

Out of a total of 24 (51.06%) patients, 13 (27.66%) did not have a capsule invasion (expression of Ki67 varies in the range 7.62-5.77%) and 11 (23.40%) had a capsule invasion (Ki67 expression varies in the range 9.73-5.80%). For Z = 11.10 and p> 0.05 (p = 0.27) patients who had capsule invasion had a slightly higher expression of Ki67 than patients who did not have capsule invasion (Table 4).

Table 4. Capsule Invasion / Expression of Ki67

Variable	Rank Sum No	Rank Sum Yes	U	Z	p-level	Valid N No	Valid N Yes
Expression	143,50	156,50	52,50	-1,10	0,27	13	11

5. Vascular invasion

Out of a total of 24 (51.06%) patients, 23 (48.93%) had vascular invasion of the tumor and in 1 (2.13%) patient vascular invasion was not established.

In patients with vascular invasion of the tumor, the expression of Ki67 varies in the range of 8.52-5.88%.

6. Lymph node metastases

Out of a total of 24 (51.06%) patients, 15 (31.91%) did not have lymph node metastases (expression of Ki67 varies in the range 7.93±4.28%) and 9 (19.15%) had metastases in lymph nodes (expression of Ki67 varies in the range 9.67±7.81%). For Z = 0.00 and p> 0.05 (p = 1.00) There was no significant difference in Ki67 expression between patients who had or did not have lymph node metastases (Table 5).

Table 5. Lymph node metastases / Ki67 expression

Variable	Rank Sum No	Rank Sum Yes	U	Z	p-level	Valid N No	Valid N Yes
Expression	187,50	112,50	67,50	0,00	1,00	15	9

7. Tumor Type & Multifocality

The results shown in Table 6. refer to the performed cross-tabulation between tumor type and multifocal tumors. Of the 23 (48.9%) benign tumors, there was no multifocality. Of the 24 (51.1%) diagnosed papillary thyroid cancer, 18 (38.3%) had no multifocality and 6 (12.8%) had multifocal tumors. In the performed cross-tabulation between tumor type and multifocality for Fisher's Exact Test p <0.05 (p = 0.022) / Monte Carlo Sig, (2-sided) there is a significant difference.

Table 6. Tumor type & Multifocality

Yes No			Multifocality		Total
Type	Benign	Count	23	0	23
		%	48,9%	0,0%	48,9%
	Carcinoma	Count	18	6	24
		%	38,3%	12,8%	51,1%
Total		Count	41	6	47
%		87,2%	12,8%	100,0%	

8. Tumor type & / Ki67 expression

Out of a total of 47 (100.00%) operated tumors, 24 (51.06%) were diagnosed with papillary thyroid cancer (expression of Ki67 varies in the range 8.58-5.76%) and 23 (48.94%) were benign changes in the thyroid gland (expression of Ki67 varies in the range 1.61±1.23%). For Z = 5.49 and p <0.001 (p = 0.000) the cancers have significantly higher expression of Ki67 than benign tumors (Table 7).

Table 7. Tumor type & / Ki67 expression

Variable	Rank Sum Carcinoma	Rank Sum Benign	U	Z	p-level	Valid N Carcinoma	Valid N Benign
Expression	834,00	294,00	18,00	5,49	0,000	24	23

DISCUSSION

Thyroid carcinomas mainly develop from follicular cells, only medullary carcinoma develops from parafollicular cells. Papillary carcinomas belong to the group of well-differentiated thyroid cancers. Papillary carcinoma is the most common primary tumor of the thyroid gland and accounts for 70% to 80% of all thyroid cancers. It is more common in women (the ratio of women to men is 2-4: 1) and is less aggressive. However, biological behavior of the tumor is not always as such. Part of papillary thyroid carcinoma can manifest itself in an aggressive nature such as the presence of cervical lymph node metastases, recurrence of the disease itself, distant metastases and even death [16].

Ki67 is a type of protein that acts in DNA binding and is present during cell proliferation. As a marker of cell proliferation, a number of studies are investigating its use in the treatment of tumors. It is mainly located in the cell nucleus and plays an important role in maintaining a stable DNA structure during mitosis. Ki67 has become an important indicator of tumor cell proliferation activity. As Ki67 expression increases, the proliferative activity of tumor cells also increases. Ki67 correlates with the degree of differentiation, tumor invasion, metastasis, and prognosis of many tumors. Patients with high Ki67 expression have a poor prognosis [17]. In this study, the value of Ki67 expression in papillary thyroid cancer relative to benign thyroid changes was statistically significant. Some studies also confirm that Ki67 expression is useful in the differential diagnosis of papillary thyroid cancer [18].

This study showed that Ki67 expression in papillary thyroid cancer is more evident, and correlates with multifocality. While capsule invasion, tumor size, and patients younger than 45 years had slightly greater expression of Ki67, there was no correlation with sex, cervical lymph node metastases or tumor position. With increasing tumor size and multifocality, the intensity of expression and the positive rate of Ki67 are evidently increased.

We demonstrate that expression of Ki67 is increased in papillary thyroid cancer. Expression of Ki67 in papillary carcinoma has been associated with tumor size and multifocality.

CONCLUSION

Ki67 is an appropriate biomarker used to distinguish papillary carcinoma from benign thyroid lesions. Ki67 expression was associated with tumor size and multifocality. High expression of Ki67 could be an important indicator for assessing the clinical course and prognosis of the disease itself.

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