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RESEARCH ARTICLE

An Experimental Study for Ki67 in Papillary Thyroid Cancer: A Single-center Experience

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ABSTRACT

Objective: The objective of this study was to determine the role of Ki67 expression level in papillary thyroid carcinoma (PTC).

Materials and Methods: This is a retrospective study of patients undergoing a thyroidectomy. A total of 150 PTC or benign thyroid disease patients were retrospectively analyzed. Cytological and immunohistochemical evaluations of Ki67 were based on the material, obtained by fine-needle aspiration biopsy. Proliferative activity was immunohistochemically assessed. The obtain ki67 values were compared with the postoperative pathological findings. Other clinicopathological factors were identified through statistical analyses.

Results: There were 86 patients in the PTC group and 74 patients in the benign group (thyroid adenomas, nodular thyroid disease, and thyroiditis). One hundred and thirty-five of the patients were female and 15 were male. The median age was 48 years (range 29–74). The Ki67 expression level in the PTC group was significantly higher than benign thyroid disease group (p=0.010). The cutoff value for Ki67 in ROC analysis was determined as 31.2 (%) with a sensitivity of 67.4% and a specificity of 62.2% (area under the curve: 0.668). However, there was no significant relationship between Ki67 expression level in the PTC group and clinicopathological factors.

Conclusion: Ki67 may be a useful biomarker in distinguishing PTC from benign thyroid disease. Nevertheless, longer follow-up studies with large samples are needed to better determine the relationship between Ki67 and clinicopathological findings.

Keywords: Benign thyroid diseases, Ki67, papillary thyroid carcinoma, thyroidectomy

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INTRODUCTION

Since the 1990s, the incidence of thyroid carcinoma has been increasing with the effect of environmental factors.[1,2] At present, it is known that the most common carcinoma of endocrine cancers is thyroid carcinoma.[3,4] The majority of papillary thyroid cancers have a good prognosis. In some patients with aggressive tumors, the prognosis is quite poor. Fine-needle aspiration biopsy (FNAB) is the gold standard made with ultrasonography in preoperative diagnosis. However, the rate of detection of malign histopathology varies between 4 and 30% in the literature and unnecessary thyroidectomy is performed. Therefore, genetic and molecular studies such as telomerase promoter mutations, BRAF V600E mutation, Ki67, or thyroglobulin are increasing in recent years to increase diagnostic accuracy.[5–8] Ki67 is a DNA binding protein that is mainly distributed in the nucleus and is related to cell proliferation. In the literature, cases with poor prognosis in breast and prostate cancer are reported to have high expression of Ki67.[9]

The role of Ki67 on papillary thyroid cancer and thyroid diseases is an attractive area and still studies were exploring the unknown significance. In this study, we investigated the effect of Ki67 on PTC and benign thyroid disease as well as on the clinical and pathological values in PTC patients.

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MATERIALS and METHODS

In this retrospective study, 150 formalin-fixed, paraffin-embedded surgical specimens of lobectomies, thyroidectomies, and excision biopsies were retrieved from the archives of the department of pathology over a period of 4 years. The study was conducted after obtaining approval from the Institutional Review Board (No. KAEK/2020.05.6). There were 74 cases of nonneoplastic lesions (multinodular goiter, thyroiditis, and others) and 86 cases of neoplastic lesions (papillary thyroid carcinomas [PTC]). Cytological and immunohistochemical evaluations of Ki67 were based on the material, obtained by FNAB. Proliferative activity was immunohistochemically assessed. The obtain Ki67 values were compared with the postoperative pathological findings.

Total thyroidectomy was performed in all patients. Prophylactic central lymph node dissection was not routinely performed for PTC patients in our hospital. Lymph node dissection was planned in patients with suspicious lymph node involvement for metastasis in neck US imaging. All specimens were examined by experienced pathologists. Tumor multifocality, extrathyroidal extension, capsule invasion, lymphovascular invasion, and lymph node metastasis were recorded. Multifocality was considered when two or more tumor foci were observed in the same lobe of the gland. Tumor size was accepted as the dominant nodule diameter. The clinical pathological staging was based on 2014 National Comprehensive Cancer Network guidelines. Furthermore, the age and sex of patients were recorded.

Ki67 expression was determined by immunostaining with monoclonal antibodies. Monoclonal antibodies to Ki67 were purchased from Cell Marque Company Limited in California, United States. Paraffin-embedded tissue samples were used. Immunohistochemical analysis was performed using bond polymer to refine detection on an automated Leica Bond Max instrument. Epitope retrieval was performed with 20 min in EDTA. Protocol peroxide block; 10 min, marker; 25 min, post primer; 8 min, polymer; 8 min, and DAB refines; made in 6 min. All slide images were obtained by scanning the 3DHistech Panoramic 250 Flash III Scanner at 40× size and saved in mrxs file format. Ki67 counting was done with Virapath Ki67 Algorithm digital image analysis program. The digital analysis program was carried out by a specialist pathologist in tumor areas classified as benign and malignant. The anodized areas were determined to cover at least half of the tissue remaining around the tumoral region. In the small tissues, all tissues around the tumor area were studied. The fields in the tables are also specified in mm square. Lymphocytes and other inflammatory cells were not separated by the analysis program and were not included in the count. Ki67 positivity index was calculated automatically by the algorithm as the ratio of the number of ki67 positive cells to the total number of cells.

Statistical Analysis

All patients in the study were recorded. SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The descriptive statistics for data were presented as mean and standard deviation values. The conformity of the numerical variables to the normal distribution was examined using the Shapiro-Wilk test. To investigate the differences between two independent groups, non-parametric Mann–Whitney U-test was used since the normality assumption was violated. Receiver operating characteristic (ROC) curves were used to determine the optimal cutoff values of the Ki67 expression intensity. $P<0.05$ was considered statistically significant.

RESULTS

There were 150 cases in the study group, including 15 males and 135 females. The median age was 48.5 (range min: 29, max: 74) years.

The intensity of Ki67 expression in PTC and benign nodular disease groups is shown in Table 1. The expression density of Ki67 in the PTC group was significantly higher than the group of benign thyroid disease ($p=0.010$). In the ROC analysis was shown in Figure 1, the cutoff value for Ki67 was determined as 31.2 (%) with 67.4% sensitivity and 62.2% specificity (area under the curve: 0.668).

Table 2 summarizes the clinical and pathologic characteristics of the PTC patients. There were 78 women (89.8%) and eight men (10.2%) in the PTC group. The mean age was 47.11±12.66 years. The mean tumor size in the PTC group was 1.57±1.38 cm. Eight (10.2%) of PTC patients had extrathyroidal extension. Multifocality was detected in 22 patients (34.3%) independently. Lateral lymph node metastasis with central lymph node was present in four patients (4.8%). With the PTC group, clinical and pathological characteristics were compared according to Ki67 expression intensity. Clinical and pathological factors are listed in Table 2. Ki67 expression density was not statistically significant in tumors less than 1 cm compared to 1 cm and above.

In our study, Ki67 expression intensity was higher in the PTC group than benign thyroid disease group ($p<0.05$). However, Ki67 expression density did not show a statistically significant difference in the PTC group based on clinicopathological subgroups ($p>0.05$).

Table 1. Comparison of Ki67 expression density between benign thyroid disease group and papillary thyroid carcinoma

Group	Case (n)	Expression intensity (%)	p
Papillary thyroid carcinoma	86	60.973±53.159	**0.010**
Benign thyroid disease	74	32.964±23.902	

DISCUSSION

For many years, pre-operative diagnostic fine-needle aspiration biopsy combined ultrasound has been used to differentiate benign or malignant thyroid nodules.[10–12] In clinical practice, most centers frequently use metastatic lymph nodes as a surrogate of malignancy. Although this approach was considered to have high precision compared, it still could not differentiate malignancy in some clinical scenarios.[13,14] Based on this factor, researchers have made extensive efforts to diagnose thyroid cancer and to verify their prognosis, and in recent years, they focus on to find trustworthy biomarkers such as Ki67 which was a DNA binding protein that is mainly distributed in the nucleus and is related to cell proliferation, also one of the important markers of cell proliferation.[15] Furthermore, the structure of Ki67 was similar to that of some proteins involved in cell cycle regulation.[16] Ki67 did not exist in calm cells (G0), but begins to appear in the core in G1 phase. Subsequently, in the S and G2 phases, Ki67 protein expression increases gradually along the M phase and peaks, followed by a rapid decrease over the M phase.[17,18] The expression of Ki67 is closely related to tumor cell proliferation and growth and is widely used as a proliferation marker in routine pathology studies. It is widely known that high Ki67 expression is associated with poor prognosis in breast cancer and prostate cancer.[19,20]

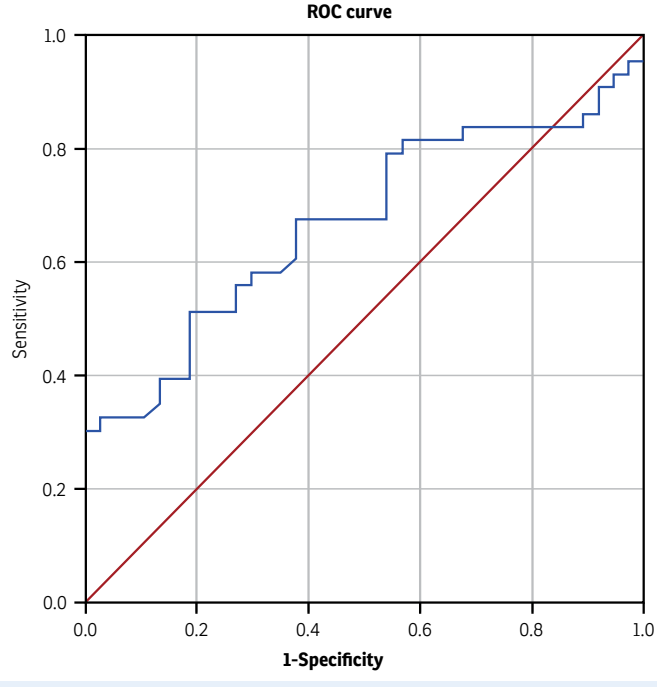

Figure 1. ROC analysis of Ki67 expression density in the study group

ROC: Receiver operating characteristics

Table 2. Evaluation of clinical and pathological features in papillary thyroid carcinoma (n=86)

Parameters	Case (n)	Expression intensity (%)	p
Gender			
Male	8	30.850±57.498	0.181
Female	78	43.550±53.322	
Age			
<45 years	38	50.370±67.504	0.582
>45 years	48	34.977±35.921	
Tumor size			
<1 cm	36	43.750±58.258	0.887
>1 cm	50	41.370±50.329	
Multifocality			
Yes	22	59.613±31.962	0.401
No	64	57.090±52.198	
Extrathyroidal extension			
Yes	8	98.686±84.112	0.550
No	78	51.161±49.372	
Lymph node metastasis			
Yes	4	56.146±26.022	0.268
No	82	57.805±57.202	
Capsule invasion			
Yes	22	58.866±38.684	0.773
No	64	87.410±50.983	
Lymph vascular invasion			
Yes	14	45.672±16.202	0.292
No	72	60.068±51.203	

Ki67 levels of either benign or malignant thyroid disease vary in the literature. Wallin et al.[21] revealed that Ki67 has no role in differentiating between benign and malignant lesions. They observed a Ki67 LI of 0–1.1% in non-neoplastic lesions, 0–3.1% in benign lesions, and 0.2–3.9% in malignant lesions. In addition to these data, in several retrospective single institution

series, no significant difference in Ki67 level was observed for histological typing of PTC and/or other thyroid tumors and benign thyroid diseases such as Graves' disease.[22,23] In contrast to this finding, Horii et al.[24] assessed Ki67 using flow cytometry in 17 benign and 33 malignant thyroid tumors. The percentage of Ki67 was 39.9±3.9 (%) in malignant tumors and found to be significantly higher than benign tumors, which was 9.4±2.1 (%). Ziad et al.[25] studied immunoexpression of thyroid transcription factor-1 (TTF-1) and Ki67 in cancer pathological specimens and found a significantly higher Ki67 LI (30±5) in anaplastic areas in comparison with the follicular areas (2±1). They suggested that in thyroid cancers, TTF-1 and Ki-67 could provide useful information on the differentiation activities of thyroid tumor cells and may be helpful to distinguish well-differentiated and undifferentiated areas in a mixed thyroid cancer. Our Ki67 levels were 60.973±53.159 (%) for PTC and 32.964±23.902 (%) for benign lesions, the difference was high and was found to be statistically significant (p=0.010).

Tumor size is a well-recognized prognostic factor for PTC as well as multifocality, extrathyroidal extension, tumor size, and lymph node metastases.

Tang et al.[26] found that in tumors larger than or equal to 1 cm only, Ki67 expression density was statistically higher than in tumors smaller than 1 cm and tumor size was linearly related to Ki67 expression density. In contrast to this result, no correlation was found Ki67 between tumor size in our study. Considering the number of samples in this sense, the number of samples in our study was not as adequate as in the other two studies.

The limitations of the present study were the small sample size (n=150) comprising a limited number of cases of PTC and benign nodular disease. In this study, it was studied only in fine-needle biopsy series and did not include other thyroid cancers. As it is an experimental study, it cannot be generalized to the whole population. However, the main strength of our study was the pathological examination that was performed by only one pathologist, and this mineralized the variability's on Ki67 assessment. Despite the above-mentioned limitations, Ki67 was found to be helpful to PTC from benign nodular disease. As our study includes only small, numbers of patients, larger studies were considered necessary to confirm this observation.

CONCLUSION

Our study confirmed that Ki67 was highly expressed PTC and could be used to differentiate PTC from benign thyroid disease. The expression intensity of Ki67 in PTC was not associated with prognostic factors. In this study, the relationship between Ki67 and PTC was first addressed in the Turkish population. Consequently, Ki67 cannot be used as a single predictive factor in determining papillary thyroid cancer and determining its behavior. A combination of some factors should be used to increase the accuracy of detection of malignancy. Based on this result, we were planning to design a prospective data collection study to explore whether addition of thyroglobulin and BRAF mutation results could improve the importance of Ki67 in these population. Ki67 should be tested combined with another biomarker.

Disclosures

Ethics Committee Approval: The study was approved by the University of Health Sciences, Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee (No: KAEK/2020.05.6, Date: 15/05/2020).

Informed Consent: Written informed consent was obtained from all patients.

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