Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.01.057 2019;26(4):728-33

Original Article

Comparison of PTEN expression in Hashimoto thyroiditis, follicular adenoma, papillary and follicular carcinomas

Saadet Alan, Nese Karadag Soylu

Inonu University, Faculty of Medicine, Department of Medical Pathology, Malatya, Turkey

Abstract

Aim: Thyroid malignancies account for approximately 3% of all human cancers. A loss or reduction in the expression of a tumor suppressor gene, PTEN has been observed in approximately 40% of thyroid tumors. This change in PTEN expression has been shown to be due to PTEN mutation or deletion. The aim of this study was to compare the PTEN expression among Hashimoto thyroiditis, follicular adenomas and malignant tumors originating from thyroid follicle epithelial cells.

Material and Methods: 101 cases of thyroid carcinoma were studied and classified into differentiated types including 15 cases of follicular thyroid carcinomas (FTC), 28 cases of papillary thyroid carcinomas (PTC), 29 cases of follicular adenomas (FA) and 29 cases of Hashimoto thyroiditis (HT). PTEN expression in all the cases were analyzed immunohistochemically.

Results: The cytoplasmic and nuclear staining intensity in Hashimoto thyroiditis showed minimal loss of PTEN expression. The nuclear and cytoplasmic staining intensity and percentage in Hashimoto thyroiditis and follicular adenomas were similar. In most of the papillary carcinoma samples, PTEN expression was lost as deduced from cytoplasmic and nuclear staining intensity. While the loss of nuclear PTEN expression was the highest in follicular carcinoma, the cytoplasmic loss was minimal.

Conclusions: Loss of PTEN expression is more pronounced in papillary and follicular carcinomas than benign lesions. PTEN has been shown to play an oncogenic role in papillary and follicular carcinoma. PTEN expression loss can be used as a new biomarker in PTC and FTC cases.

Keywords: Hashimoto Thyroiditis; Follicular Adenom; Papillary Thyroid Carcinoma; Follicular Thyroid Carcinoma; PTEN.

INTRODUCTION

Thyroid cancers constitute approximately 3% of all malignant tumors. In recent years, the incidence of thyroid carcinomas is significantly increased (1,2). The majority of thyroid carcinomas (87.9%) are papillary thyroid carcinoma (PTC), less frequently follicular (FTC), medullary (MTC) and anaplastic carcinoma (ATC) (3). Diagnosis of carcinoma is made by radiological imaging and cytopathological examination after aspiration cytology. The development of multifocal carcinoma in multiple areas of the thyroid parenchyma simultaneously supports the aetiology of PTC. The only evidence-based etiologic factor in the pathogenesis of thyroid cancer is ionizing radiation (4). The association of autoimmune lymphocytic thyroiditis with PTC was demonstrated in cross-sectional studies. The relationship between PTC and HT has also been supported by recent cross-sectional studies (2,5-8). Although the process of PTC formation in HT has been described by faulty regeneration in the follicular epithelium after chronic inflammatory injury, the molecular pathogenesis remains unclear. The presence of HT correlates with multifocal cancers as reported by many studies (5,9-11).

The tumor suppressor gene PTEN, also known as MMAC1 and TEP-1, is mutated frequently in many types of cancer (12). Suppression of PTEN expression leads to progression and increased invasiveness of thyroid cancer (12-14). PTEN expression reduction in benign and malignant lesions of thyroid has been reported. There is no study comparing benign lesion/adenomas of thyroid with well-differentiated malignant follicular epithelial tumors (4). In this study, we aimed to compare the relationship between carcinogenesis and PTEN expression in papillary and follicular carcinomas and benign lesions of thyroid (Hashimoto thyroiditis, follicular adenomas). The relationship between benign and malignant lesions will

Received: 29.01.2019 **Accepted:** 02.03.2019 **Available online:** 11.03.2019
Corresponding Author: Saadet Alan, Inonu University, Faculty of Medicine, Department of Medical Pathology, Malatya, Turkey
E-mail: saadet.alan@inonu.edu.tr

facilitate detection and discrimination in the early stages of the process, which would help with early intervention and treatment.

MATERIAL and METHODS

The pathology archive scanning was carried out in Inonu University, Faculty of Medicine, Turgut Özal Hospital, Department of Medical Pathology. A total of 101 cases with 15 follicular carcinomas, 28 papillary carcinomas, 29 follicular adenomas, and 29 Hashimoto thyroiditis were randomly selected. The Medical Ethics Committee of Inonu University approved the study.

Macroscopic features (size, location) and clinical anamnesis (age, gender) of the patients were reported. The pathological specimens retrieved from the archive have been re-evaluated. The parafin tissue blocks for immunohistochemical staining were selected in all cases. The blocks were sectioned into 4-5 µm thickness and the streptavidin biotin peroxidase immunohistochemical staining method was performed to evaluate PTEN immunoexpression in the tissue. The sections were treated with 3% hydrogen peroxide for 20 min to suppress endogenous peroxidase activity. The nonspecific background was blocked by serum-free protein block. Then sections were incubated with PTEN primary antibodies (Mob 369, clone 28H6 isotype IG G1, Kappa, CA) at room temperature for 1 h followed by three washings in PBS. The kit was used for detection of protein according to the manufacturer's instructions. Mayer's hematoxylin was used to counterstain the cells. A negative control (no primary antibody) was performed. Coverslips were mounted on glass slides using mounting media. Slides were blinded, and three random fields were digitized using a Olympus BX51 microscope attached to a digital camera.

In immunohistochemical evaluation, PTEN expression was evaluated in endothelial cells as internal positive control. Slides were evaluated by considering the prevalence of nuclear and cytoplasmic stainings. Nuclear and cytoplasmic staining density signal in thyroid were rated as strong (+++), medium (++), weak (+) and negative -). Nuclear and cytoplasmic staining was classified under percentage of cells showing staining as 0-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4). Finally, the staining density score was calculated. The degree of staining density and the percentage of staining were multiplied. These values were evaluated as 0, negative (-); 1-4, weak positive (+); 5-8, moderate positive (++) and 9-12, strong positive (+ ++) as previously reported in the literature (6). Data were evaluated by using SPSS 11.0 commercial statistics program. The difference in PTEN expression among the groups was evaluated using Kruskal Wallis, Mann Whitney U and Spearman's correlation test in all groups. $p<0.05$ was taken to indicate statistical significance.

RESULTS

In the study, 15 of the samples were follicular carcinomas (FTC), 28 were papillary carcinomas (PTC), 29 were follicular adenomas (FA) and 29 were Hashimoto thyroiditis (HT) (Figures 1A-B-C-D). The age of the patients ranged from 20-74 years and the mean age was 46±13 years. 78% of the cases were female (mean age 46±14), 23% were male (mean age 45±11). The distributions of age, sex, tumor size of FTC, PTC, FA, HT groups were shown in Table 1.

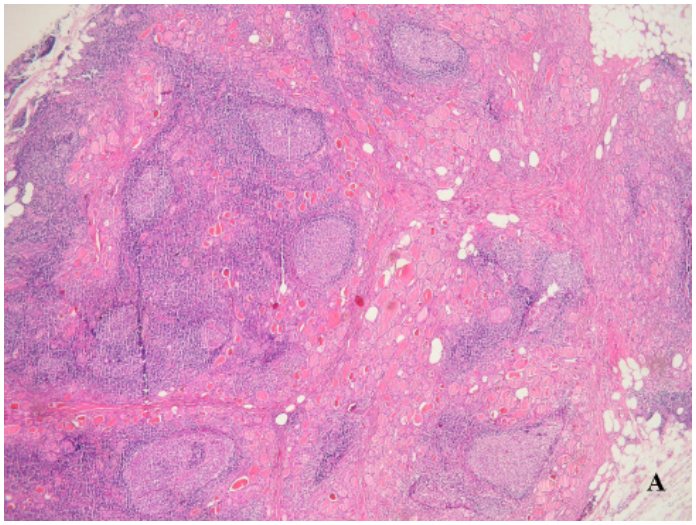

Figure 1A. Hashimoto's thyroiditis (x40, HE)

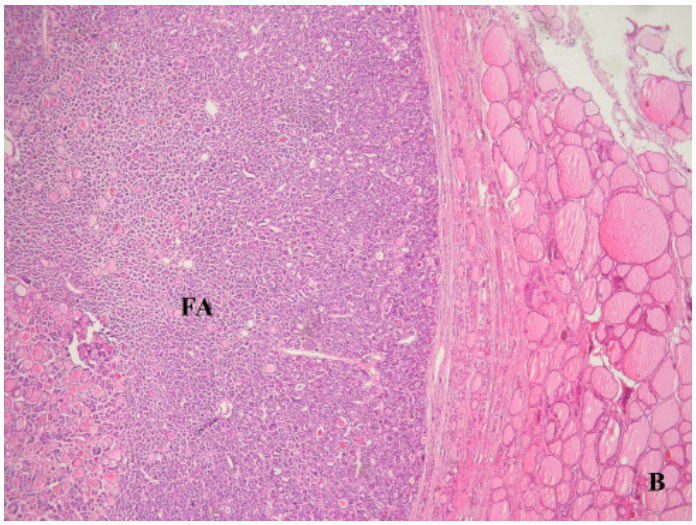

Figure 1B. Follicular adenoma (x40,HE)

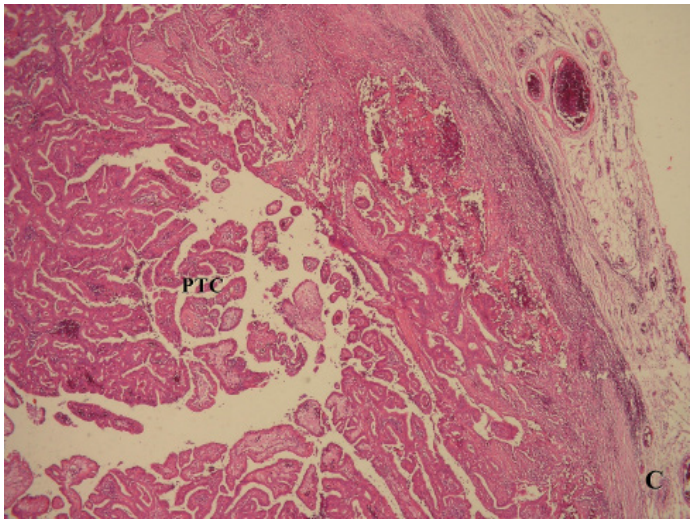

Figure 1C. Papillary carcinoma (x40, HE)

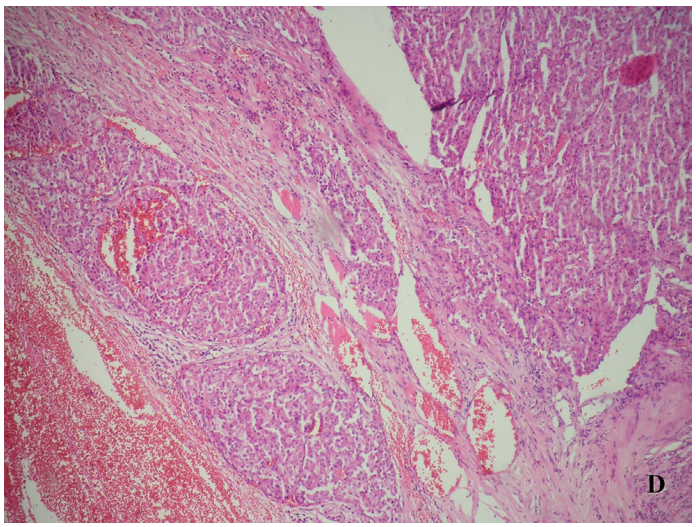

Figure 1D. Vascular invasion in follicular carcinoma (x100, HE)

Table 1. Age, gender, tumor size distributions of the cases. a,b,cMeans bearing different superscripts within same column were significantly different (P <0.05)

	HT (n=29)	FA (n=29)	PTC (n=28)	FTC (n=15)
Age	26-72, 44±2	26-74, 45±2	23-74, 48±3	20-74, 43±4
Gender				
Female	86%, 44±2	62%, 45±3	75%, 47±3	80%, 42±5
Male	14%, 44±9	38%, 46±2	25%, 53±5	20%, 50±3
Tumor size (months±Std. Error of Mean)		1.2-12 (4± 0.4)[a]	0.3-11.5 (1.9± 0.4)[b]	1.5-8.3 (3.9±0.5)[a]

The tumor size of PTC is significantly smaller than that of FTC, FA tumor groups (p<0.001). There was no correlation between tumor size, gender and age of FTC, PTC and FA groups (r <1). In our study, when all of the cases were examined together, the tumors were localized in 47.2% of cases in the right lobe, in 38.9% of them in the left lobe, in 4.2% of them in isthmus, in 9.7% of them in right+left+isthmus lobe. Hashimoto thyroiditis was present in all of the cases (Figure 2A). In comparison to other groups in terms of nuclear staining density, it was observed that staining was stronger in Hashimoto thyroiditis group (p<0.001). In 5 of these cases, cytoplasmic staining was also observed. In Hashimoto thyroiditis, there was a minimal loss of expression as assessed by cytoplasmic density. The nuclear staining was detected in 19 cases; cytoplasmic staining in 3 cases; and both nuclear and cytoplasmic stainings were detected in 6 cases of follicular adenomas. In follicular adenoma, more moderate nuclear staining intensity was observed (Figure 2B).

In comparison to other groups in terms of nuclear staining density, it was observed that staining was stronger in Hashimoto thyroiditis group (p<0.001). In 5 of these cases, cytoplasmic staining was also observed. In Hashimoto thyroiditis, there was a minimal loss of expression as assessed by cytoplasmic density.

Moderate nuclear staining was observed more frequently in follicular and papillary carcinoma (Figures 2C, 2D). There was no difference among the follicular carcinomas, papillary carcinomas and follicular adenomas in terms of nuclear staining density (p>0.05).

Papillary carcinoma and follicular adenoma has shown diffuse nuclear staining and there was no difference in the prevalence of nuclear staining among these groups (p>0.05). In follicular carcinoma cases, the prevalence of nuclear staining was weaker. When follicular carcinoma was compared with papillary carcinoma, follicular adenoma and Hashimoto thyroiditis groups, the prevalence of nuclear staining was found to be significantly different (p<0.05).

The loss of expression in follicular carcinoma in terms of cytoplasmic prevalence was found to be less than in other groups. There was no significant difference between groups in terms of cytoplasmic density and prevalence (p>0.05). In all groups, the mean and expression values of the staining density and the prevalence of nuclear-cytoplasmic PTEN expression are shown in Table 2.

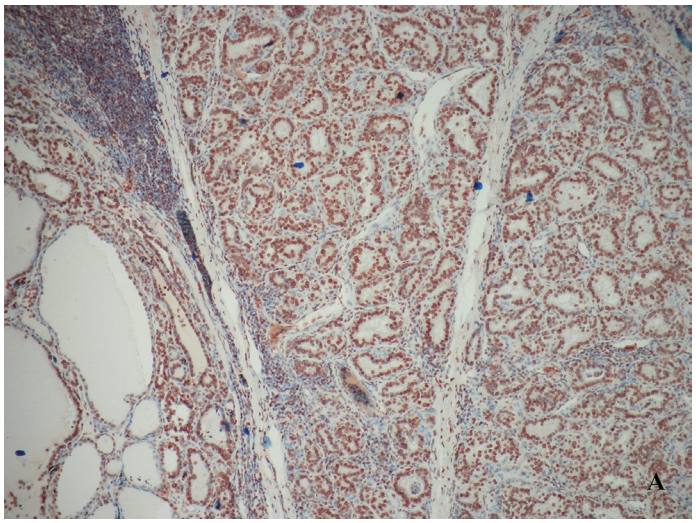

Figure 2A. Diffuse, strong nuclear positivity in Hashimoto's thyroiditis with PTEN (x100, IHK)

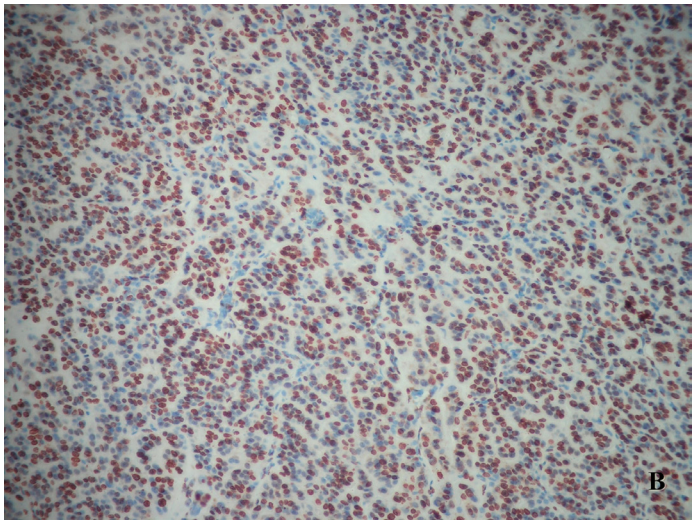

Figure 2B. Diffuse, strong nuclear positivity in follicular adenomas with PTEN (x200, IHK)

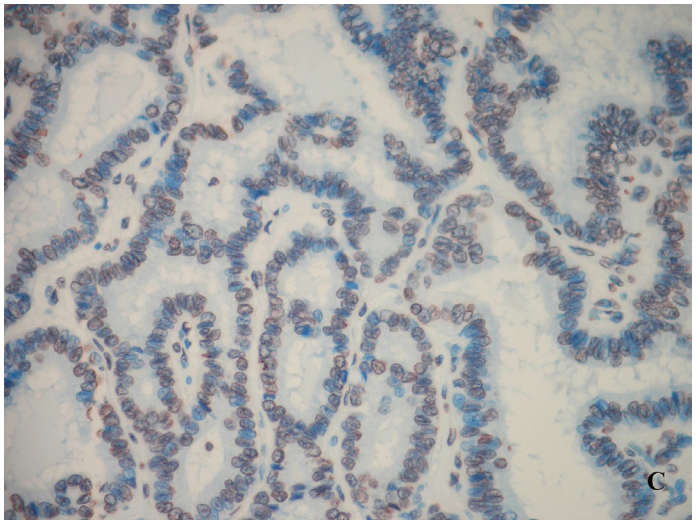

Figure 2C. Papillary carcinoma shows weak, patchy nuclear positivity with PTEN (x200, IHK) Figure 2D. Follicular carcinoma shows weak, patchy nuclear positivity with PTEN (x200, IHK)

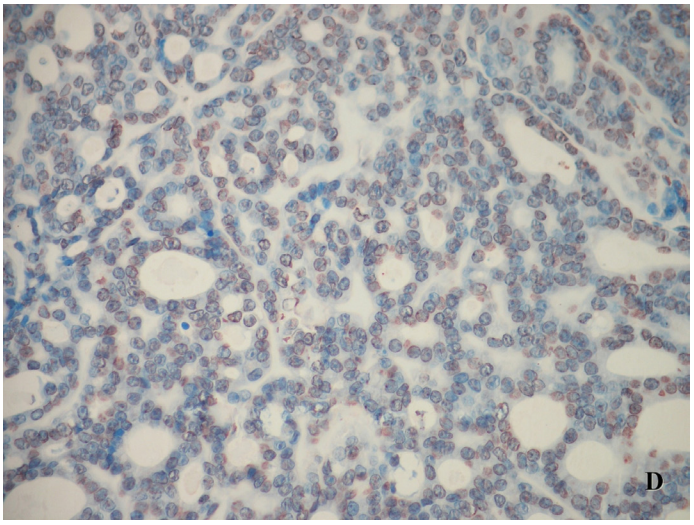

Figure 2D. Follicular carcinoma shows weak, patchy nuclear positivity with PTEN (x200, IHK)

Table 2. Nuclear density and nuclear prevalence values in terms of PTEN expression of the cases. a, b, c Means bearing different superscripts within same column were significantly different (P <0.05).

	HT (n=29)	FA (n=29)	PTC (n=28)	FTC (n=15)
Nuclear density	2.76 ± 0.08[b]	2.19±0.15a	2.04 ± 0.13[a]	2.13±0.13[a]
Nuclear prevalence (A.M±S.D)	3.00 ± 0.14[a]	2.92±0.17[a]	3.00 ± 0.15[a]	2.40±0.19[b]
Cytoplasmic density	2.20 ± 0.3[b]	1.90 ± 0.2[b]	1.40 ± 0.4[a]	2.00 ± 0.0[b]
Cytoplasmic prevalence (A.M ± S.D)	2.00 ± 0.4[a]	1.90 ± 0.3[a]	2.40 ± 0.7[b]	2.80 ± 0.6[b]
Nuclear expression	+3	+2	+2	+2
Cytoplasmic expression	+2	+1	+1	+2

DISCUSSION

Papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) are classified as well-differentiated thyroid cancers accounting for approximately 95% of all thyroid cancers. They generally have a favorable prognosis (2, 15). Şamdancı et al. reported that the incidence of PTC has been detected in New York, USA, 10.4%; Tikva, Israel, 5%, Milan, Italy, 18.5%, Saudi Arabia, 8.6%, Pakistan, 3.1% and Malatya, 21% (16).

In our study, the mean age of the patients was 46±13 years. 78% of the cases were female (mean age 46±14), 23% were male (mean age 45±11). Lin et al. reported that the mean age of patients with 6219 thyroid nodules was 49.7±13.6 (17). In a study of 14.545 patients with papillary carcinoma by Podnos et al. the mean age of the patients was 45 years (18). Fujimoto et al. found as 51 years in women and 41 years in men mean age of the patients in PK cases consisting of 363 patients (19). Corapcıoglu et al. in their study on 44 patients with thyroid papillary microcarcinoma (PMC) was reported the average age as 49 (range 20-71) (20). The results of our study are consistent with the literature (21,22). In our study, there were 15 PMC cases and the smallest tumor size was measured as 0.3 cm. Among the follicular carcinoma, papillary carcinoma and follicular adenoma cases, the largest tumor size was 12 cm and the smallest tumor size was 0.3 cm (average tumor size of 3.2±0.3 cm). There was a statistically significant difference in the tumor diameter of papillary carcinoma group as compared to other groups (p <0.001) (Table 1). No correlation was reported between age and gender with tumor diameter in the literature. In our study, there was no relationship between and age and gender with tumor diameter. Corapcioglu et al. reported that tumor was localized in the 72% of right lobe, 24% of left lobe and 4% of isthmus in the thyroid PMC patients (20). While the tumor was more localized in the left lobes in patients with follicular carcinoma, it was more localized in the right lobes in patients with papillary carcinoma and follicular adenomas.

PI3K/Akt signaling pathway activates chemokine receptors and promotes leukocyte migration. It is effective in maintaining the balance between cell survival and apoptosis. PI3K inhibitor, PTEN is expressed in normal thyroid follicular epithelial cells. Hashimoto is also expressed in thyrocyte affected thyroiditis, but is suppressed in PTC transformed epithelial cells (4). The loss or reduction of PTEN expression is observed in thyroid neoplasms. Decreased PTEN expression was reported in about 40% of thyroid tumors (23-25). PTEN expression is decreased in carcinomas with loss of differentiation. Defective PTEN protein expression observed in thyroid cancers is often not due to mutations in the PTEN gene.

The decrease in PTEN expression is due to the decrease in protein and mRNA levels at the transcriptional level of the PTEN gene (24).

PTEN protein expression has been reported to be generally localized in the nucleus and rarely in the cytoplasm of thyrocytes (25). Oliver et al. showed that nuclear PTEN (6H2,1 monoclonal antibody) immunostaining in thyroid carcinomas is weaker than normal thyroid follicle cells and follicular adenomas (24). The nuclear expression in benign thyroid tumors was relatively weaker than normal thyroid follicle cells. A study reported that follicular carcinomas have stronger PTEN expression than papillary carcinomas and undifferentiated carcinomas (24).

Oliver et al. has shown strong PTEN expression in normal thyroid epithelium (24). The staining was more nuclear and less cytoplasmic. In benign thyroid tumors, a weaker nuclear expression than normal follicular cells was observed, cytoplasmic staining remained unchanged. In contrast, nuclear and cytoplasmic PTEN immunexpression decreased in thyroid carcinomas. The decrease in nuclear staining density is more pronounced than the decrease in cytoplasmic density, especially in FTC and PTC. The decrease in cytoplasmic PTEN immunoreactivity in the transition from normal cells to differentiated and undifferentiated tumors show the inactivation of PTEN in thyroid tumorigenesis. The decrease in PTEN expression results in a reduction of apoptosis and cell-cell adhesion control (25-28).

In our study, it was observed that the loss of PTEN expression in the papillary carcinoma group was the highest in terms of nuclear and cytoplasmic density. In Hashimoto thyroiditis, the loss of expression in terms of nuclear and cytoplasmic density was minimal. While loss of PTEN expression is the highest in follicular carcinoma in terms of nuclear prevalence, the loss of expression is minimal in terms of cytoplasmic prevalence. The prevalence and intensity of nuclear and cytoplasmic staining were similar in follicular adenomas and Hashimoto thyroiditis. Loss of PTEN expression is more prominent in follicular and papillary carcinomas compared to other benign lesions.

Follicular carcinoma, papillary carcinoma, and follicular adenoma were mostly seen in moderate nuclear staining intensity. According to other groups, Hashimoto's thyroiditis has a stronger intensity of nuclear staining. Papillary carcinoma, follicular adenoma, and Hashimoto's thyroiditis show widespread nuclear staining. The prevalence of nuclear staining is weaker in follicular carcinoma cases. When cytoplasmic density and prevalence of all groups were compared, no significant difference was found.

CONCLUSION

Loss of PTEN expression is more pronounced in papillary and follicular carcinomas than benign lesions. PTEN has been shown to play an oncogenic role in papillary and follicular carcinoma. PTEN expression loss can be used as a new biomarker in PTC and FTC cases.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: This retrospective study was made by the approval of Inonu University Human Local Ethical Committee (Protocol no:2006-65)

Saadet Alan ORCID: 0000-0003-2329-151X
Nese Karadag Soylu ORCID: 0000-0003-0886-8122

REFERENCES

1. Kong Y, Yin J, Fu Y, et al. Suppression of Elk1 inhibits thyroid cancer progression by mediating PTEN expression. Oncology Reports 2018;40:1769-76.
2. Resende de Paiva C, Grønhøj C, Feldt-Rasmussen U, et al. Association between Hashimoto's thyroiditis and thyroid cancer in 64,628 Patients. Front Oncol 2017;7:53.
3. Lee JC, Sidhu SB. Papillary thyroid cancer: the most common endocrine malignancy. Endocrinology Today 2013;2:15-20.
4. Molnár C, Molnár S, Bedekovics J, et al. Thyroid carcinoma coexisting with Hashimoto's Thyreoiditis: Clinicopathological and molecular characteristics clue up pathogenesis. Pathol Oncol Res 2019;21.
5. Lee JH, Kim Y, Choi JW, et al. The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis. Eur J Endocrinol 2013;168:343-9.
6. Kurukahvecioglu O, Taneri F, Yüksel O, et al. Total thyreoidectomy for the treatment of Hashimoto's thyroiditis coexisting with papillary thyroid carcinoma. Adv Ther 2007;24:510-6.
7. Tamimi DM. The association between chronic lymphocytic thyroiditis and thyroid tumors. Int J Surg Pathol 2002;10:141-6.
8. Huang BY, Hseuh C, Chao TC, et al. Well-differentiated thyroid carcinoma with concomitant Hashimoto's thyroiditis present with less aggressive clinical stage and low recurrence. Endocr Pathol 2011;22:144-9.
9. Pisanu A, Piu S, Cois A, et al. Coexisting Hashimoto's thyroiditis with differentiated thyroid cancer and benign thyroid diseases: indications for thyroidectomy. Chir Ital 2003;55:365-72.
10. Kebebew E, Treseler PA, Ituarte PH, et al. Coexisting chronic lymphocytic thyroiditis and papillary thyroid cancer revisited. World J Surg 2001;25:632-7.
11. Consorti F, Loponte M, Milazzo F, et al. Risk of malignancy from thyroid nodular disease as an element of clinical management of patients with Hashimoto's thyroiditis. Eur Surg Res 2010;45:333-7.
12. Razavi MH, Modarressi P, Yaghmaei SM, et al. Circulating levels of PTEN and KLLN in papillary thyroid carcinoma: can they be considered as novel diagnostic biomarkers? Endocrine 2017;57:428-35.
13. Beg S, Siraj AK, Jehan Z, et al. PTEN loss is associated with follicular variant of Middle Eastern papillary thyroid carcinoma. British J Cancer 2015;112:1938-43.
14. Xu J, Cai J, Jin X, et al. PIG3 plays an oncogenic role in papillary thyroid cancer by activating the PI3K/AKT/PTEN pathway. Oncology Reports 2015;34:1424-30.
15. Larson SD, NJ Lindsey, Taylor SR. Increased incıdence of well-differentiated thyroid cancer associated with Hashimoto's Thyroiditis and the role of the PI3K/Akt pathway. J Am Coll Surg 2007;204:764-75.
16. Şamdancı E, Şahin N, Aydın NE. Histopathologic subtypes of surgically resected papillary thyroid carcinomas in Malatya region. HYPERLINK "http://www.totmdergisi.org/archive.php" \t "_top"Journal of Inonu University Medical Faculty 2010;17:77-80.
17. Lin JD, Hsuen C, Chen JY, et al. Cystic change in thyroid cancer. Anz J Surg 2007;77:450-4.
18. Podnos YD, Smith DD, Wagman LD, et al. Survival in patients with papillary thyroid cancer is not affected by the use of radioactive isotope. J Surg Oncol 2007;96:3-7.
19. Fujimoto Y, Obara T, Yamashita T. Papillary thyroid carcinoma: Rationale for hemithyroidectomy and regional node dissection. In:Clark OH, Duh OY. Textbook of endocrine Surgery. 1st edition 2003;82-93.
20. Corapcioglu D, Sak SD, Delibasi T, et al. Papillary microcarcinomas of the thyroid gland and immunohistochemical analysis of

expression of p53 protein in papillary microcarcinomas. J Transl Med 2006;4:28.
21. Rosai J. Rosai and Ackerman's. Surgical Pathology. 9th edition. Mosby, China 2004;515-68.
22. DeLellis RA, et al. Pathology and genetics of tumours of endocrine organs. WHO Classification of Tumours, 3rd Edition 8 IARC 2004;49-133.
23. Lionel ML, Suzanne JB. PTEN function in normal and neoplastic growth. Cancer Letters 2006;241:184-96.
24. Oliver G, Aurel P, et al. Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. American Journal of Pathology 2000;156:1693-700.
25. Carla DL, Gianluca T, et al. PTEN and Egr-1 expresion in thyroid proliferative lesions. Cancer Letters 2005;224:105-9.
26. Bruni P, Boccia A, et al. PTEN expression is reduced in a subset of sporadic thyroid carcinomas: evidence that PTEN-growth suppressing activity in thyroid cancer cells mediated by p27kip1. Oncogene 2000;19:3146-55.
27. Frisk T, Foukakis T, Dwight T, et al. Silencing of the PTEN tumor-suppressor gene in anaplastic thyroid cancer. Genes Chromosome Cancer 2002;35:74-80.
28. Weng LP, Gimm O, et al. Transient ectopic expression of PTEN in thyroid cancer cell lines induces cell cycle arrest and cell type-dependent cell death. Human Mol Genet 2001;10:251-8.