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ORIGINAL ARTICLE

The relationship between types of inflammatory cells located at the micro-environment of papillary thyroid microcarcinoma prognostic factors

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Summary

Purpose: *To determine the relationship between inflammatory cell types in the microenvironment of papillary thyroid microcarcinoma (PTMC) and prognostic factors.*

Methods: *The previous diagnoses and subtypes-variants of 163 patients with papillary thyroid microcarcinoma were re-evaluated according to the 2017 WHO classification. The peritumoral lymphocyte, plasma cell, neutrophil, eosinophil, and mast cell density were classified as none (0.24 mm^2), mild (0-10/0.24 mm^2), moderate (10-50/0.24 mm^2), and severe (>50/0.24 mm^2) under 40x magnification and the relationship with prognostic factors was investigated.*

Results: *There was a statistically significant relationship between tumor capsule invasion (p=0.024) and surgical margin (p=0.049) with mast cell infiltration. A statistically significant relationship was observed between tumor capsule invasion (p=0.0001) and the postoperative disease-free period (p=0.0001) with neutrophil cell infiltration. The postoperative disease-free period of those with neutrophil infiltration was statistically significantly shorter than that of those with no infiltration. The tumor diameter of those with no plasma cells was statistically significantly smaller than that of patients with plasma cells (p=0.003).*

Conclusions: *Closer follow-up of patients with neutrophils, mast cells, and plasma cells, which have been found to be associated with poor prognostic factors in terms of recurrence, lymph node involvement, and distant metastasis, may increase survival.*

Key words: *inflammatory cells, papillary microcarcinoma, prognostic factors, thyroid, survival*

Introduction

Papillary thyroid microcarcinoma (PTMC) is defined by the World Health Organization (WHO) as a specific subgroup of papillary thyroid carcinoma (PTC) with a maximum tumor diameter of 1.0 cm [1]. In 2008, the incidence of PTMC was 11.8% in the total population and the prevalence was reported to be 36% in several autopsy series [2,3]. PTMCs are generally slow-growing tumors and most can be surgically removed. Together with the popularization of ultrasonography (USG) and fine-needle aspiration biopsy (FNAB), the rate and fineness of samples in histopathologic examinations increased for preoperative surgical specimens and the rate of postoperative PTMC detection increased [1,2,4,5-12]. Although PTMC is considered as indolent cancer with relatively benign biologic behavior and perfect prognosis, prognostic factors such as insufficient resection, lymph node metastasis (e.g. lymph node enlargement over 3mm, multiple lymph node involvements), distant metastasis (e.g. pulmonary, bone), extra-capsular involvement, multicentricity, and recurrence may cause an ag-

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gressive course or even lead to death [1,2,6-8,12]. The reported mortality rate of PTMC varies between 0% and 1%. The characteristic cytologic features of PTMC are useful in diagnosing specimens after the FNA or surgical resection [3,6,7,12], some have papillary arrangements, nuclear clarification, well-circumscribed nuclear membranes, micronucleoli, intranuclear cytoplasmic inclusions, nuclear grooves, and psammoma bodies [9,12] (Figure 1a).

Chronic infection and inflammation, which are held responsible in the etiology of various tumors, contribute to 25% of all cancer cases worldwide [7,10]. Many biochemical mechanisms that change during chronic inflammation are involved in tumorigenesis. These mechanisms include the shift of the cellular redox balance to oxidative stress, stimulation of genomic instability, an increase of DNA damage, stimulation of cellular proliferation, metastasis, and angiogenesis, the release of epigenetic control of gene expression, and inappropriate epithelial-mesenchymal transition (EMT). Various pro-inflammatory cytokines, prostaglandins, nitric oxides, and matricellular proteins are closely related with the premalignant and malignant transformation of the cells in the background of chronic inflammation. Inflammatory mediators induce genomic instability and disturb DNA repair mechanisms. Inflammation causes EMT and accelerates metastasis [11].

Although a direct connection between various gastrointestinal system malignancies and inflammation types has been established, the relationship with PTC remains controversial. In addition, many epidemiologic and morphologic studies reported increased PTC risk among patients with Hashimoto thyroiditis (HT) [13] (Figure 1b).

In the present study, the relationship of inflammatory cell types and distribution in the microenvironment of tumors with several histomorphologic and prognostic factors was examined to detect tumors earlier by considering previous studies on PTMC.

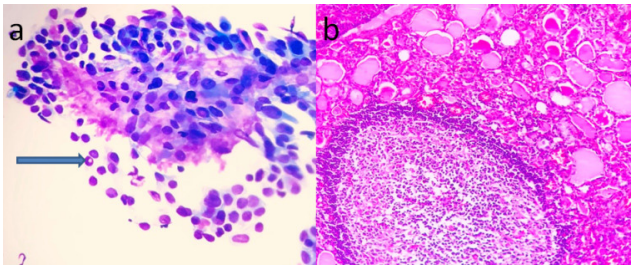


Figure 1. a: PTMC cytologic sample consisting of papillary structures with fibrovascular cores in the central parts; cells have dense squamoid cytoplasm, enlarged overlapping nuclei with irregular contours, intranuclear pseudoinclusions (indicated by arrow) and grooves, and pale, fine granular chromatin (FNA, Giemsa stain x 40), **b:** Hurthle cell metaplasia, lymphoid elements and germinal center-prominent lymphoid follicular structure in Hashimoto's thyroiditis (HEx40).

Methods

Study design

One hundred sixty-three patients who were diagnosed with PTMC in the Pathology Department of Usak University Training and Research Hospital and the Pathology Department of Meram Medical Faculty of Necmettin Erbakan University between 2013 and 2019 were retrospectively analyzed in the present study. The previous diagnoses and subtypes-variants of the 163 patients with PTMC were re-evaluated according to the 2017 WHO classification and the final decisions were considered in this study.

In PTMCs, peritumoral lymphocytes (PTL), plasma cells, neutrophils, eosinophils, and mast cell density were classified as none (0.24 mm^2), mild (0-10/0.24 mm^2), moderate (10-50/0.24 mm^2), and severe (>50/0.24 mm^2) under 40x magnification, Giemsa histochemical stain was applied to the cases to detect mast cells more easily and the relationship with prognostic factors such as tumor diameter, multicentricity, lymphovascular-

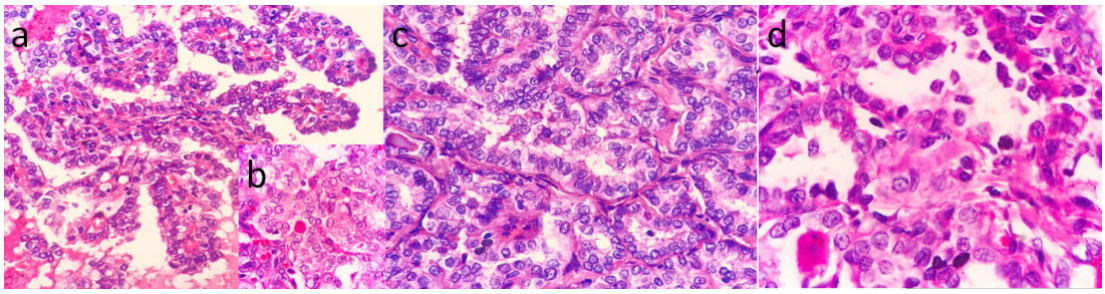


Figure 2. a: Classic variant PTMC sample consisting of papillary structures with fibrovascular cores in the central parts (H&E X40), **b:** Tumor cells in Classical variant PTMC sample show nuclear enlargement, slight contour irregularity, intranulear pseudoinclusion, groove and fine granular chromatin structure (Inset), **c:** Follicular variant PTMC; neoplastic cells arranged as macro or microfollicles with central colloid (H&E X40), **d:** Oncocytic variant PTMC; Neoplastic cells with large eosinophilic-granulated cytoplasm, papillary carcinoma nuclear features (H&E X100)

perineural invasion, tumor capsule invasion, surgical margin involvement, and lymph node metastasis and the presence of HT were examined.

Statistics

The data were analyzed using the SPSS statistical software package, version 22. If the variables obtained had normal distribution the Shapiro-Wilk test was used due to the number of units. In interpreting the results, statistical significance was set at 0.05 ($p<0.05$ indicated that the variables did not have normal distribution, whereas $p>0.05$ indicated that the variables had normal distribution). In examining the intragroup differences, the Mann-Whitney U test was used because the variables were not normally distributed. Chi square analysis was used to examine the relationships between nominal variables.

Results

Among the patients with lymphocytic infiltration, lymphovascular invasion, tumor capsule invasion, multicentricity, perineural invasion, and surgical margin invasion were observed at low frequencies. A statistically significant relationship was found between sex and PTMC subtypes ($p=0.038$). Dominance of female sex was observed in PTMC subtypes (classic, follicular, and oncocytic) (Figure 2a,b,c). 135 (82.8%) of the patients were female and 28 (17.2%) male. The age of patients ranged between 22 and 75 years (mean: 48.6). The survival period ranged between 1 and 317 months (mean: 16.16).

Among 86 patients with severe lymphocytic infiltration, 36 (41.8%) had HT, 11 (12.7%) had tumor capsule invasion, two (2.3%) had perineural invasion, and 4 (4.6%) had surgical margin positivity. Regarding inflammatory cell infiltration, 58.3% had severe infiltration, 30% moderate, and 9.8% had mild lymphocytic infiltration; no infiltration was observed in 2%. Plasma cell infiltration was severe in 3% of the patients, medium in 49%, and mild in 29.5%; no plasma cell infiltration was seen in 18.5% of the patients. Severe and moderate infiltration was not observed with eosinophil and mast cells, whereas mild infiltration was observed in 1.3 to 29.5%, respectively (Figures 3,4,5,6). Capsule invasion was observed in 5.5% of patients with neutrophilic infiltration. Of our cases, 40.4% had neutrophilic infiltration at a minimal level (<10/0.24 mm^2) and 2.06% at a moderate level (10-50/0.24 mm^2). It was observed that the infiltration was at the center of/close to the tumor and intratumoral (Figure 7). However, no patients were found to have severe infiltration (>50/0.24 mm^2).

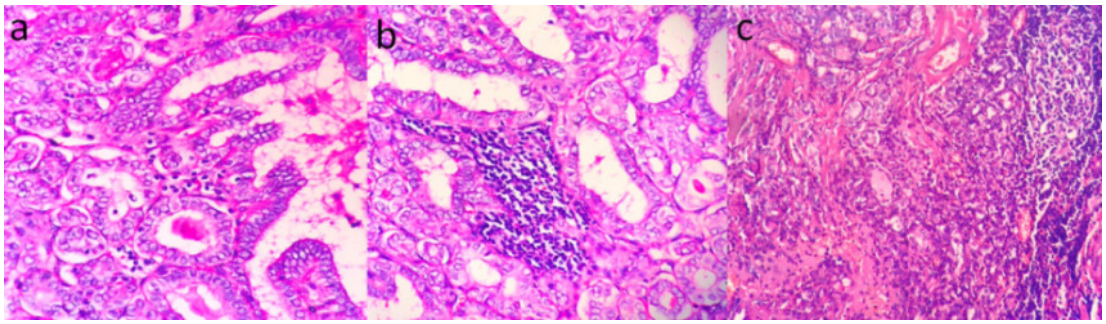


Figure 3. a: Minimal lymphocytic infiltration, **b:** moderate lymphocytic infiltration, **c:** severe lymphocytic infiltration (H&E x40).

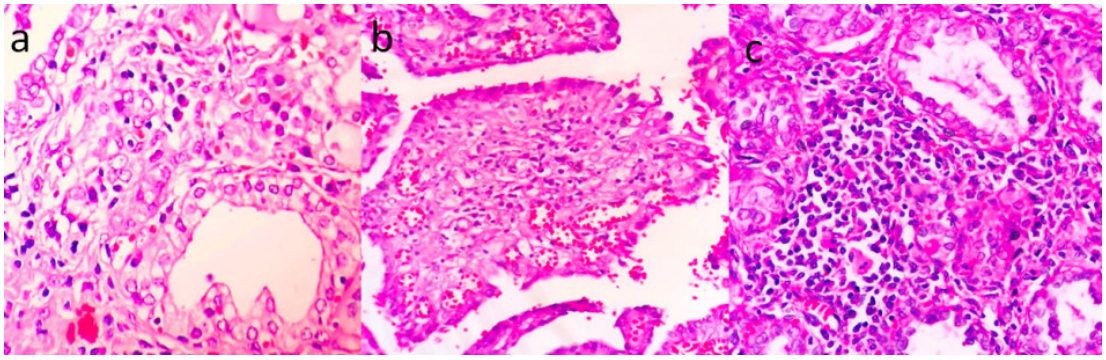


Figure 4. a: Minimal plasma cell infiltration (indicated by arrow) (H&E x40), **b:** moderate plasma cell infiltration (indicated by arrow) (H&E x40), **c:** severe plasma cell infiltration (indicated by arrow) (H&E x40).

The rate of tumors located in the right lobe and left lobe was 43.6% (n=71) and 38.7% (n=63), respectively, the rate of tumors with right + left localization was 13.5% (n=22), and the rate of isthmus localization was 4.3% (n=7). There was a statistically significant relationship between sex and PMC tumor subtype (p=0.038). It was determined that 26.8% of patients with follicular type of PTMC, 11.1% with classic type PTMC, and 25% of patients with other types of PTMC were male, whereas 73.2% of patients with follicular type PMC, 88.9% with classic type PTMC, and 75% of those with other types of PTMC were female.

A statistically significant relationship was found between plasma cell and PTMC subtypes (p=0.002). The plasma cell type was detected in 67.9% of patients with follicular type PTMC, in 89.9% of patients with classic type PTMC, and in 87.5% of patients with other types of PTMC (Table 1). A statistically significant relationship was found between tumor capsule invasion and surgical margin invasion with mast cell infiltration (p=0.024, p=0.049). Among the patients with mast cell infiltration, 8.3% were found to have capsule invasion and 10.4% had surgical margin invasion (Table 2). The presence of neutrophils had a statistically significant relationship with tumor capsule invasion and accompanying tumor (p=0.001, p=0.039). Among the patients with neutrophilic infiltration, 30.1% had capsule invasion and 9.6% had accompanying tumors (Table 3). A statistically significant relationship was found between the post-operative disease-free period and neutrophil infiltration (p=0.0001) (Table 4). The postoperative disease-free period of patients with neutrophil infiltration was statistically significantly shorter than that of patients with no infiltration. A statistically significant relationship was found between HT and plasma cell infiltration (p=0.005). HT was found in 30.6% of the patients with plasma cell infiltration (Table 5). Tumor diameter had a statistically significant relationship with plasma cell infiltration (p=0.003). The diameter of tumors in patients with no plasma cell infiltration was significantly smaller than those of patients with plasma cell infiltration (Table 6).

Of the patients included in the present study, 79.1% (n=129) underwent a total and 14.1% (n=23) a subtotal thyroidectomy, whereas 2.4% (n=4) underwent left lobectomy and 4.2% (n=7) underwent right lobectomy. In the 1-5-year postoperative follow-up of 163 patients, atypical lymph nodes were detected in one (0.6%) patient's USG. Three (1.8%)

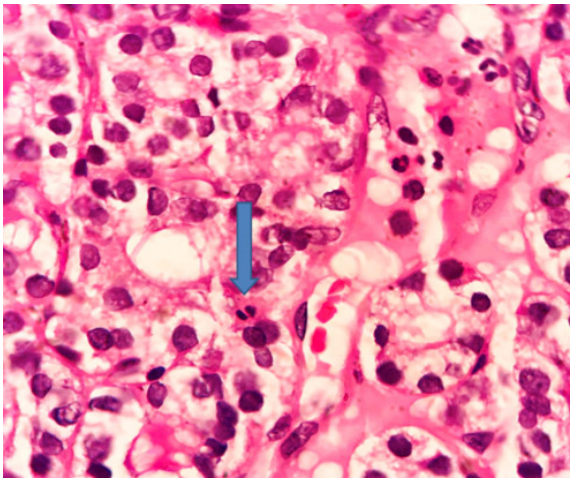


Figure 5. Minimal eosinophilic infiltration (indicated by arrow) (H&E x100).

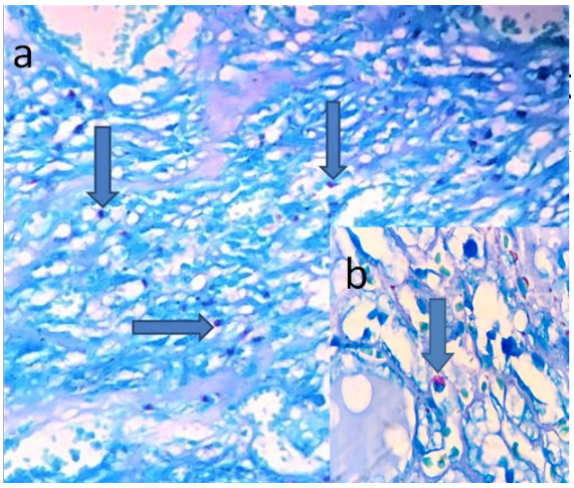


Figure 6. a-b: Minimal mast cell infiltration in histochemical staining of Giemsa (indicated by arrow) (H&E x40, H&E x100).

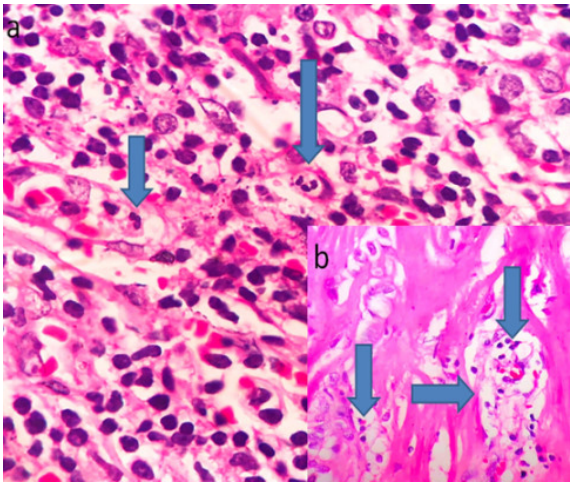


Figure 7. a: Minimal neutrophilic infiltration (indicated by arrow) (H&E x100), **b:** moderate neutrophilic infiltration (indicated by arrow) (H&E x40).

patients were not followed up and 160 (98.1%) were observed to have no recurrence or atypical lymph node. The smallest tumor diameter was 0.35 mm and the largest 9 mm, and the mean diameter was 5.5 mm. Multicentricity was found in 54 (33.1%) of the 163 patients, among whom, tumor foci were observed to be located in the same lobe in 32 (59.2%) patients and at a different lobe in 21 (38.8%). No mortality occurred during follow-up. Inflammatory cell grades in the tumors are shown in Graph 1.

Table 1. Chi-square test result regarding the relationship between PTMC type and variables

Variables	*PTMC sub tumor type*				*Chi-square analysis*
	PTMC Follicular type n (%)	*PTMC Classic type n (%)*	*PTMC Oncocytic type n (%)*	*Total n (%)*	*p*
Sex					0.038
Male	15 (26.8)	11 (11.1)	2 (25.0)	28 (17.2)	
Female	41 (73.2)	88 (88.9)	6 (75.0)	135 (82.8)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Tumor location					-
Isthmus	3 (5.4)	4 (4.0)	0 (0.0)	7 (4.3)	
Right lobe	21 (37.5)	49 (49.5)	1 (12.5)	71 (43.6)	
Left lobe	27 (48.2)	31 (31.3)	5 (62.5)	63 (38.7)	
Right-Left lobe	5 (9)	15 (15.2)	2 (25.0)	22 (13.5)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
TmCI					-
Capsule invasion	19 (33.9)	21 (21.2)	1 (12.5)	41 (25.2)	
No capsule invasion	21 (37.5)	41 (41.4)	3 (37.5)	65 (39.9)	
Without capsule	16 (28.6)	37 (37.4)	4 (50.0)	57 (35.0)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Multicentric					0.281
Absent	42 (75.0)	62 (62.6)	5 (62.5)	109 (66.9)	
Present	14 (25.0)	37 (37.4)	3 (37.5)	54 (33.1)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Eosinophils					0.183
Absent	50 (89.3)	77 (77.8)	7 (87.5)	134 (82.2)	
Present	6 (89.3)	22 (22.2)	1 (12.5)	29 (17.8)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Mast cells					0.606
Absent	42 (75.0)	67 (67.7)	6 (75.0)	115 (70.6)	
Present	14 (25.0)	32 (32.3)	2 (25.0)	48 (29.4)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Lymphocytes					-
Absent	4 (7.1)	0 (0.0)	0 (0.0)	4 (2.5)	
Present	52 (92.9)	99 (100.0)	8 (100.0)	159 (97.5)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Neutrophils					-
Absent	39 (69.6)	46 (46.5)	5 (62.5)	90 (55.2)	
Present	17 (30.4)	53 (53.5)	3 (37.5)	73 (44.8)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	
Plasma					0.002
Absent	18 (32.1)	10 (10.1)	1 (12.5)	29 (17.8)	
Present	38 (67.9)	89 (89.9)	7 (87.5)	134 (82.2)	
Total	56 (100.0)	99 (100.0)	8 (100.0)	163 (100.0)	

TmCI: tumor capsule invasion, PNI: perineural invasion, SMI: surgical margin invasion, HT: Hashimoto thyroiditis.

Discussion

In a study by Kuo et al [13], it was reported that no lymphocytic infiltration was detected in 52% of tumors, but it was shown to be related with multicentric and lymph node metastasis, the classic histologic characteristics of PTC with lymphocytes. It was determined that the overall survival of patients with cancer with lymphocytic infiltration was longer (log-rank p=0.018). Some researchers, however, reported that thyroid cancer with tumor-related lymphocytes showed a worse disease course and increased invasion and lymph node metastasis frequency [7,14-17].

In the present study, no significant relationship was found between the variables and the presence of lymphocytic infiltration (p>0.05). Lymphocytic infiltration was observed in the vast majority (97.5%) of patients and severe infiltration (>50/0.24 mm^2) was observed in 55.8%. The vast majority of patients with severe lymphocytic infiltration in the present study were females (78.8%), and lymphovascular invasion, tumor capsule invasion, multicentricity, perineural invasion, and surgical margin invasion were observed at lower levels among those with severe lymphocytic infiltration.

The association between thyroid cancers (especially PTC) and HT has been reported in many studies since 1995 and the rate of comorbidity was reported to vary between 0.5% and 38% [14-16]. In a study by Segal et al [18], it was reported that patients with thyroid carcinoma accompanied by HT had a better prognosis and autoimmune inflammatory reaction and the presence of antibodies in circulation prevented the growth and extension of thyroid carcinoma.

One hypothesis is that the tissue damage occurring as a result of the immune response in HT might play a role in the etiopathogenesis of PTMC [14]. Jeong et al [16] found that the accompany-

Table 2. Chi-square test results on the relationship between mast cell status and variables

Variables	*Mast cells*			*Chi-square analysis*
	Absent n (%)	*Present* n (%)	*Total* n (%)	*p*
Sex				0.734
Male	21 (18.3)	7 (14.6)	28 (17.2)	
Female	94 (81.7)	41 (85.4)	135 (82.8)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	
TmCI				0.024
Capsule invasion	27 (23.5)	14 (29.2)	41 (25.1)	
No capsule invasion	53 (46.1)	12 (25.0)	65 (39.9)	
Without capsule	35 (30.4)	22 (45.8)	57 (35.0)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	
Multicentrite				0.343
Absent	80 (69.6)	29 (60.4)	109 (66.9)	
Present	35 (30.4)	19 (39.6)	54 (33.1)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	
PNI				0.632
Absent	112 (97.4)	46 (95.8)	158 (96.9)	
Present	3 (2.6)	2 (4.2)	5 (3.1)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	
SMI				0.049
Absent	112 (97.4)	43 (89.6)	155 (95.1)	
Present	3 (2.6)	5 (10.4)	8 (4.9)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	
HT				0.637
Absent	87 (75.7)	34 (70.8)	121 (74.2)	
Present	28 (24.3)	14 (29.2)	42 (25.8)	
Total	115 (100.0)	48 (100.0)	163 (100.0)	

TmCI: tumor capsule invasion, PNI: perineural invasion, SMI: surgical margin invasion, HT: Hashimoto thyroiditis.

Table 3. Chi-square test results on the relationship between neutrophil status and variables

Variables	*Neutrophils*			*Chi-square analysis*
	Absent n (%)	*Present* n (%)	*Total* n (%)	*p*
Sex				0.999
Male	15 (16.7)	13 (17.8)	28 (17.2)	
Female	75 (83.3)	60 (82.2)	135 (82.8)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
TmCI				<0.001
Capsule invasion	19 (21.1)	22 (30.1)	41 (25.1)	
No capsule invasion	48 (53.3	17 (23.3)	65 (39.9)	
Without capsule	23 (25.6)	34 (46.6)	57 (35.0)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
Multicentrite				0.149
Absent	65 (72.2)	44 (60.3)	109 (66.9)	
Present	25 (27.8)	29 (39.7)	54 (33.1)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
PNI				0.657
Absent	88 (97.8)	70 (95.9)	158 (96.9)	
Present	2 (2.2)	3 (4.1)	5 (3.1)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
SMI				0.141
Absent	88 (97.8)	67 (91.8)	155 (95.1)	
Present	2 (2.2)	6 (8.2)	8 (4.9)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
HT				0.091
Absent	72 (80.0)	49 (67.1)	121 (74.2)	
Present	18 (20.0)	24 (32.9)	42 (25.8)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	
Accompanying tumors				0.039
Absent	76 (84.4)	66 (90.4)	142 (87.1)	
Present	14 (15.6)	7 (9.6)	21 (12.9)	
Total	90 (100.0)	73 (100.0)	163 (100.0)	

TmCI: tumor capsule invasion, PNI: perineural invasion, SMI: surgical margin invasion, HT: Hashimoto thyroiditis.

Table 4. Mann-Whitney U test result regarding the difference between neutrophil status in terms of values

Variables	*Neutrophils*						*Mann-Whitney U test*		
	n	*Mean*	*Median*	*Minimum*	*Maximum*	*SD*	*Average Rank*	*z*	*p*
Age								-0.681	0.496
Absent	90	50	50	22	79	13	84.27		
Present	73	48	50	23	76	12	79.21		
Tumor diameter								-1.2	0.221
Absent	90	4.63	4.25	0.35	9.00	2.37	77.94		
Present	73	5.09	5.00	0.80	9.00	2.39	87.01		
Focus number								-0.27	0.787
Absent	90	2	2	2	5	1	27.00		
Present	73	2	2	2	6	1	27.93		
Post operative disease free time								-4.2	0,0001
Absent	90	18	15	2	42	10	93.71		
Present	73	12	10	1	47	10	62.12		

ing HT was related with better prognostic factors such as younger age, smaller tumor size, lower rate of recurrence, female sex, lower stage, longer survival, and lower extra-thyroidal extension. In the present study, HT was detected in 25.8% of patients with PTM and this rate is within the limits reported in the literature. Although this result corroborates the perspective that HT frequently accompanies the cases with PTMC, no significant relationship was found in the present study (p=0.111). Severe lymphoid cell infiltration and lymphoid follicle infiltration were observed in 45 patients and accompanying HT was detected in 30 (66.6%).

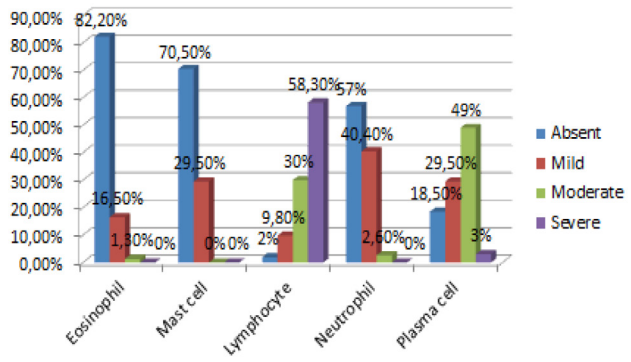
Table 5. Chi-square test results regarding the relationship between plasma status and variables

Variables	*Plasma*			*Chi-square analysis*
	Absent n (%)	*Present* n (%)	*Total* n (%)	*p*
Sex				0.999
Male	5 (17.2)	23 (17.2)	28 (17.2)	
Female	24 (82.8)	111 (82.8)	135 (82.8)	
Total	29 (100.0)	134 (100.0)	163 (100.0)	
Multicentric				0.698
Absent	18 (62.1)	91 (67.9)	109 (66.9)	
Present	11 (37.9)	43 (32.1)	54 (33.1)	
Total	29 (100.0)	134 (100.0)	163 (100.0)	
PNI				0.587
Absent	29 (100.0)	129 (96.3)	158 (96.9)	
Present	0 (0.0)	5 (3.7)	5 (3.1)	
Total	29 (100.0)	134 (100.0)	163 (100.0)	
SMI				0.353
Absent	29 (100.0)	126 (94.0)	155 (95.1)	
Present	0 (0.0)	8 (6.0)	8 (4.9)	
Total	29 (100.0)	134 (100.0)	163 (100.0)	
HT				0.005
Absent	28 (96.6)	93 (69.4)	121 (74.2)	
Present	1 (3.4)	41 (30.6)	42 (25.8)	
Total	29 (100.0)	134 (100.0)	163 (100.0)	

PNI: perineural invasion, SMI: surgical margin invasion, HT: Hashimoto thyroiditis.

Table 6. Mann-Whitney U test results regarding the difference between plasma status in terms of values

Variables	*Plasma*						*Mann-Whitney U test*		
	n	*Mean*	*Median*	*Minimum*	*Maximum*	*SD*	*Average Rank*	*z*	*p*
Age								-0.397	0.691
Absent	29	48	49	22	77	14	78.84		
Present	134	50	50	25	79	12	82.68		
Tumor diameter								-3.01	0.003
Absent	29	3.63	3.50	0.35	9.00	2.26	58.09		
Present	134	5.10	5.00	0.80	9.00	2.34	87.18		
Focus number								-0.696	0.486
Absent	29	3	2	2	5	1	29.86		
Present	134	2	2	2	6	1	26.90		
Post- operative disease free time								-0.101	0.921
Absent	29	16	12	3	46	11	79.22		
Present	134	16	12	1	47	11	80.17		



Graph 1. Degree of density of inflammatory

There is strong evidence indicating that, in tumor models, mast cells play a determinant role in causing the angiogenic key preceding the malignant transformation and thus, they have strong effects on the progression and growth of human cancers [19]. Besides playing a primary role in the allergic innate and adaptive immune response and angiogenesis, there are also publications reporting that the mast cells may accompany various malignancies such as thyroid cancer [20-22]. In many studies, a strong relationship was detected between the presence of mast cells and the tumor stage, prognosis (especially poor prognosis), and invasion [20]. Proietti et al [23] determined that mast cells were at a remarkably higher level in intratumoral and peritumoral regions of follicular variant papillary thyroid carcinoma (FVPTC) and the mast cell density could be used in distinguishing benign and malignant forms of follicular thyroid lesions. In the present study, mast cells were detected in 32.3% of classic variants of PTC and 25% of FVPTC cases, and the presence of mast cells was found to have a statistically significant relationship with tumor capsule invasion (p=0.024) and surgical margin lesion (p=0.049). Thus, in our study, a statistically significant relationship was detected between poor prognostic factors and mast cell presence. Also, in our study, mast cell infiltration was not found in 70.5% of patients, but in the remaining 29.5%, it was detected as mild. None of the patients were detected as having moderate or severe infiltration (Graph 1).

In the literature, it was reported that the tumor microenvironment was accompanied by neutrophil and eosinophilic infiltration [13,24], and it was shown that the level of eosinophils in the peripheral blood and tissue increased in several types of cancer. Some previous studies reported that tissue or blood eosinophilia was related with significantly better prognosis, whereas other studies reported that the absence and presence of eosinophils in tumor tissue or blood were of no prognostic value. Erythropoietin (EPO) obtained from eosinophils can create synergy with reactive oxygen species in order to kill tumor cells or catalyze nitrite oxidation to create additional cytotoxic radicals [25]. In the present study, eosinophil infiltration was not observed in or around the tumor in 82.2% (n=134) of patients. In the remaining cases (17.8%, n=29), the level of infiltration was minimal (<10/0.24 mm^2). Considering the good prognosis in PTMC, it can be thought that there may be a relationship between eosinophilic infiltration and poor prognosis of thyroid carcinomas because the eosinophilic infiltration around the microenvironment of the tumor in the present study was at a minimal level.

The neutrophils were found in high amounts in both carcinoma cells and the stroma adjacent to the tumor cells, they were distributed especially at the center of tumor, and their number decreased as the distance from the tumor increased [26]. For this reason, the phenomenon of neutrophil-tumor cell emperipolesis or phagocytosis was related to poor prognosis in pleomorphic giant cell carcinoma and micropapillary carcinoma [26]. Also, neutrophils increase the turnover proliferation, favouring invasion and secreting factors that promote tumour growth [27]. In the present study, a statistically significant relationship was found between tumor capsule invasion and neutrophilic infiltration (p=0.0001) and the postoperative disease-free period (p=0.0001). The postoperative disease-free period of those with neutrophil infiltration was found to be statistically significantly shorter than for patients with no neutrophilic infiltration. Accordingly, a statistically significant relationship was detected between poor prognostic factors and the presence of neutrophilic infiltration.

In a study by Mohammed et al [28] on the microenvironment of breast cancer, it was determined that there was a positive correlation between plasma cells, other inflammatory cells, and macrophage infiltration, as well as high grade, estrogen receptor and progesterone receptor negativity, and vascular invasion. However, according to the results of a study by McIntire et al [29] on triple-negative breast cancer, a statistically significant relationship between the increase in density of plasma cell infiltration and an increase in survival rates in triple-negative breast cancer were determined. Similarly, in a study by Fristedt et al [30], it was shown that long-term survival of patients with both esophagus and gastric adenocarcinoma had a statistically significant relationship with increasing plasma cell immunoglobulin kappa C concentration. Although no relationship with the other prognostic factors was found in the present study, a statistically sig-

nificant relationship was found between tumor diameter and plasma cell infiltration (p=0.003). It was determined that tumor diameter in patients with PTC without plasma cell infiltration was significantly smaller than that of patients with plasma cell infiltration. In other words, there was a positive correlation between the presence of plasma cell infiltration and the increasing tumor diameter, which is one of the factors of poor prognosis.

In the present study, there was non-significant right lobe dominance and significant female dominance. In previous studies, it was determined that the topographic anatomic region, which is affected by the tumor the most, was the medial third of right or left thyroid lobe. The tumor showed remarkable female dominance and it is observed more frequently between the ages of 27 and 75 years [31]. It was reported in the literature that there was a relationship between the increase in thyroid cancer cases and exposure to radiation emitted by cell phones [32]. The present study has a retrospective design and it is not known for how many years and for how many hours per day the patients have been using cell phone. On the other hand, because the majority of the general population is right-handed and because of the relative dominance of the right lobe of the thyroid, it is thought that the use of cell phones perhaps may trigger the tumor.

Previous studies confirmed that the development of head-neck cancer (especially thyroid cancer) was related with long-term radiologic examinations; it was reported that high-dose treatment of small size adenocarcinomas triggered the development of thyroid cancer [33]. The Surveillance, Epidemiology, and End Result Analysis published by the United States of America National Cancer Institute showed that the incidence of thyroid cancer among patients with or without previous malignancy was higher than the incidence of other malignancies [34]. The tumors that most accompany thyroid cancer are breast and renal tumors [35]. Cybulski et al [36] determined that the mutation of CHEK2 gene, which plays a role in DNA repair of various cell types, is related with thyroid, breast, and renal cancers. Among the 163 patients in the present study, there was one case of osteochondroma, one case of adenomatous colon polyps, two cases of follicular carcinoma, one case of fibroadenoma, one case of invasive ductal carcinoma, two cases of papillary urothelial carcinoma, three cases of parathyroid adenoma, one case of colon adenocarcinoma, and one case of endometrial polyps. Among these synchronous tumors, female sex dominance was observed in all cancers, except for two cases of papillary urothelial carcinoma. As is known, PTMC is observed 4.6 times more frequently among women, and of the 163 patients in the present study, 28 (17.2%) were male and 135 (82.8%) female. This result is in parallel with the literature and the sex difference suggests that estrogens increase the proliferation of thyroid cancer cells and, thus, the incidence among women is higher when compared with men.

In a study by Apostol et al [37], 428 patients were examined in terms of sex, age, tumor size, histologic variant, localization (sub-capsular, intraparenchymal), unilateral or bilateral involvement, number of foci, lymphovascular invasion, thyroid capsule invasion, extra-thyroidal extension, and lymph node metastasis. Sub-capsular tumor location was found to have a strong relationship with lymphovascular-capsular invasion and extra-thyroidal extension. In the same way, in our study, the tumors were close to the capsule (0.1-8 mm); we think that carefully examining tumors at a close distance to the capsule in routine thyroid sampling and taking samples from these points in particular would increase the success in detecting incidental PTMC.

In a study by Seong et al [38] on 174 patients with PTC, a statistically significant relationship with cervical lymph node metastasis (CLNM) in tumors at <1.9 mm distance from the capsule in 155 patients with no capsule lesion was shown in univariate (p=0.002) and multivariate (p<0.001) analyses. Although no CLNM was found in any patient with PTC farther than 1.9 mm from the capsule, CLNM was observed in 40.8% (58/142) of patients with PTC <1.9 mm distance from the capsule. In ultrasonography, CLNM had no relationship with age, sex or tumor size. In the present study, lymphovascular invasion was detected in 4.9%, tumor capsule invasion in 25.2%, and multicentricity in 33.1%. Moreover, metastasis was observed in the lymph nodes of 17 (11.4%) patients who had already undergone lymph node excision (148 removed). The rate of tumor capsule invasion was found 21.4%. No mortality was observed.

In recent years, several clinical and histologic risks such as diameter ≥0.5 cm, multicentricity, tumor extension beyond the parenchyma, and lymph node involvement were determined as aggressiveness factors in PTMC [39]. However, the optimal size and standard treatment for these tumors remain controversial. The optimal diameter was reported as 0.7 cm by Lim et al [40], 0.6 cm by Zhang et al [41], and 0.5 cm by Chang et al [42]. In the present study, the diameters ranged between 0.35 mm and 9 mm, and the mean diameter was 5.5 mm. Multicentricity was found in almost one-third (33.1%) of 163 patients and tumor foci were observed in the same lobe in 32 (59.2%) of 54 patients showing multicentricity and in a different

lobe in 21 (38.8%). No statistically significant difference was found between multicentricity in single or multiple lobes. In general, total or subtotal thyroidectomy is recommended when tumor foci are detected in both lobes and total thyroidectomy or unilateral lobectomy is recommended when it is limited to a unilateral lobe [5,12]. In the guidelines published by the American Thyroid Association and British Thyroid Association, the treatment protocol recommended for patients with low-risk isolated PTMC is thyroid lobectomy, whereas the protocol recommended for patients older than 45 years with head and neck radiation treatment history or familial history of differentiated thyroid carcinoma in first-degree family members is total or sub-total thyroidectomy [12]. The authors recommended two treatment protocols for patients with low-risk PTMC. The first was observation and the second was emergency surgery. For follow-up, an examination after 6 months or once a year was recommended and surgical intervention was recommended if the tumor grew to 3 mm or larger or if a new lymph node metastasis was detected [6]. In the present study, total thyroidectomy was performed on the majority of patients (79.9%, n=129) because of the risk of recurrence or postoperative complication development. From a postoperative aspect, atypical lymph nodes were found after 3 months in only one (0.6%) (USG follow-up) of 163 patients.

The first limitation of the present study is that it covers a 5-year period. Also, we were unable to access information about radioactive iodine use in the patients, and we could not predict the long-term prognosis or question etiologic factors.

Conclusion

In this study, female dominance, HT coexistence, slightly increased right lobe involvement, and subcapsular tumor placement were observed. We think that more careful examination of the right lobe and subcapsular placement in sampling and taking more samples from this localization may increase the chances of identifying patients with PTMC. Also, a statistically significant relationship was found between poor prognostic factors such as capsule invasion, surgical margin positivity, large tumor diameter, postoperative short disease-free period, the presence of concomitant tumor, and neutrophil, mast cell, and plasma cell infiltration. In histopathologic examinations, we think that the inclusion of neutrophil, mast cell, and plasma cell infiltration in the high-risk group, along with closer follow-up of these patients in terms of lymph node involvement and distant metastasis, will increase the survival of these patients and prevent advanced tumors that need more severe and longer treatments.

Conflict of interests

The authors declare no conflict of interests.

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