Relationship between lymphovascular invasion and clinicopathological features of papillary thyroid carcinoma

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ABSTRACT

Lymphovascular invasion (LVI) is an important prognostic factor in various solid tumors, however, data on the association between LVI and thyroid carcinomas are limited. In this study, we evaluated the relationship between LVI and clinicopathological features of papillary thyroid carcinoma (PTC). Six hundred seventy-eight patients diagnosed with PTC between 2012 and 2015 were included into the study. Patients were classified based on the presence or absence of LVI. Gender, age, ultrasonography (US), tumor size and multifocality, BRAFV600E mutation, perineural and capsular invasion, extrathyroid extension (ETE), nodal metastasis, and recurrences were evaluated, and risk analysis was performed for each parameter. The number of patients with LVI [LVI (+)] was 63, while the number of patients without LVI [LVI (-)] was 615. The female/male ratio was 564/114. LVI was present in 18.4% of male patients and in 7.4 % of female patients. In the age group between 17-25 years LVI was detected in 6/13 patients, and this result was statistically significant compared to other age groups ($p = 0.004$). Suspicious lymph nodes upon US, perineural or capsular invasion, ETE, tumor size, and nodal metastasis were significantly more frequent in LVI (+) group ($p < 0.001$). The frequency of BRAFV600E mutation was also significantly higher in LVI (+) group ($p < 0.001$). Overall, the presence of LVI was associated with gender, tumor size, age, lymph node metastasis, pathological lymph nodes, perineural and capsular invasion, ETE, and BRAFV600E mutation. These results suggest that in PTC patients undergoing thyroidectomy, the presence of LVI should be considered as an indicator of aggressive clinicopathological features and those patients should be followed up carefully for recurrences and metastasis.

KEY WORDS: Papillary thyroid carcinoma; clinicopathological features; lymphovascular; PTC; LVI; lymphovascular invasion; BRAFV600E mutation

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INTRODUCTION

Among the tumors of endocrine system, thyroid carcinoma is the most common malignancy, although only 1% of all cancers are of this type. The majority of thyroid cancers are well-differentiated tumors originating from thyroid follicular cells. The most common type of thyroid cancer is papillary thyroid cancer (PTC), representing 85% of all thyroid cancer cases [1]. According to the data from "Cancer Facts and Figures, 2016", thyroid cancer was diagnosed in over 64,000 individuals in 2016 in the U.S.A, and the incidence rate increased approximately 5% in the last 10 years [2,3]. Although PTC is a slowly growing tumor and has an excellent prognosis, with expected 5-year survival greater than 98%, histological subtypes of PTC such as columnar cell variant (CCV), tall cell variant (TCV), and diffuse sclerosing variant (DSV) have a relatively worse prognosis [4,5]. Among the most frequently used staging systems for estimating the prognosis and risk of recurrence in thyroid carcinomas are those developed by the Union for International Cancer Control (UICC) and American Joint Commission on Cancer (AJCC) [the TNM Classification of Malignant Tumours (TNM)], European Organization for Research and Treatment of Cancer (EORTC), Mayo Clinic (Age, Grade, Extent, Size or AGES and Metastases, Age, Complete resection, Invasion, Size or MACIS), and Lahey Clinic (Age, Metastases, Extent, Size or AMES) [6]. These

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systems consistently include clinicopathological parameters, however, factors such as age, gender, tumor type and size, extracapsular extension, lymph nodes, lymphovascular invasion (LVI), and distant metastasis are not all covered by a single staging system [7-13]. Although LVI is a poor prognostic factor for numerous cancers, including laryngeal, uterine, endometrial, colorectal, cervical, bile duct, and vulvar cancer, none of the staging systems include LVI in staging of thyroid carcinomas [14-20]. In a clinical series conducted by Ieni et al. [21], 295 PTC patients (8 of them with a newly described entity - micropapillary/hobnail variant [MPHC]) were evaluated and the authors concluded that the prognosis of MPHC-PTC tumors was better compared to the other groups, possibly due to a lower rate of vascular invasion in this group [21]. In another study conducted by Xu et al. [22], extensive vascular invasion (EVI) was an independent predictor of poor recurrence-free survival in low-grade encapsulated follicular cell–derived thyroid carcinomas (LGEFCs), and patients with EVI may be considered for more aggressive treatment modalities. Data on the association between LVI and clinicopathological features of PTC are limited to small case series, and conflicting results have been reported [23-26]. In this study, we investigated the relationship between the presence of LVI and clinical features and histopathological outcomes of PTC patients.

MATERIALS AND METHODS

Medical reports of patients who were diagnosed with PTC at Faculty of Medicine, Trakya University, and operated between 2010 and 2015, were obtained from the institutional database system. The institutional Ethics Committee approved the study protocol. Patients were classified based on the presence or absence of lymphovascular invasion [LVI (+) or LVI (-)]. Gender, age, preoperative neck and thyroid ultrasonography (US), tumor size and multifocality, BRAFV600E mutation, perineural invasion, capsular invasion, ETE, lymph node metastasis, local recurrences, tumor localization, tumor focality, and the presence of Hashimoto's thyroiditis were evaluated.

Radiological examination

All patients underwent detailed preoperative neck and thyroid US for determining the presence of nodules and lymph nodes and thyroid and nodule size. Suspicious features indicating a malignant thyroid nodule, such as as microcalcification, irregular margins, ratio of anteroposterior to transverse dimension, presence of central vascularization, absence of halo, and hypoechogenicity were recorded for each nodule. The size, shape, cortex thickness, microcalcification, loss of fatty hyperechoic hilum, echogenicity, and cystic proportion were determined for lymph nodes.

Histopathological evaluation

Sections obtained from formalin-fixed, paraffin-embedded (FFPE) blocks of thyroidectomy specimens were stained with hematoxylin and eosin (H&E) stain and re-evaluated by two pathologists in a blind manner. The presence of LVI was determined if tumor cells were observed in lymphovascular spaces, the cells underlay endothelium of lymphovascular channels, the cells invaded through a vessel wall and endothelium, or thrombus was adherent to intravascular tumor, as described by Mete et al. [26]. The histopathological subtypes of PTC were classified as: classic PTC, follicular variant, oncocytic variant, and aggressive variants (CCV, TCV, and DSV). Tumor size was measured in unifocal tumors as the largest diameter and defined as "Tumor size". Each nodule for multifocal tumors was measured separately and defined as "Total tumor size".

BRAF mutation analysis

DNA was isolated from FFPE tissue samples containing at least 30% of tumor cells. Then, DNA purification was performed using nucleic acid isolation kits for FFPE tissues (QIAamp® DNA FFPE Tissue Kit, EZ1® DNA Tissue Kit, PAXgene® Tissue Containers, and PAXgene Tissue DNA Kit, QIAGEN, Hilden, Germany). Following polymerase chain reaction (PCR), pyrosequencing analyses were performed on a PyroMarkQ24 sequencing system (Hilden, Germany) using Seq Primer BRAF 600 or Seq Primer BRAF 464–469 sequencing primers.

Surgical procedure and follow-up

All patients underwent total thyroidectomy. Patients with lymph node metastasis had lymph node dissection. Thyroglobulin values, neck US, whole-body scan, follow-up frequency, and postoperative, radioiodine ablation treatment were organized case specific in accordance with American Thyroid Association Guidelines (ATA) [4].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY) and MedCalc version 12.7.7 (MedCalc Software bvba, Ostend, Belgium). Results were expressed as mean ± standard deviation (SD) or numbers and percentages. The Mann-Whitney U test was used for the comparison of numeric independent factors, including age, tumor size (unifocal), and tumor total size (multifocal), between LVI (+) and LVI (-) group. Categorical independent factors were compared using the Chi-squared test. Odds ratios and 95% confidence intervals were calculated using logistic regression analysis. For

discriminating between LVI (+) and LVI (-) groups, the cut-off values of tumor size (unifocal) and total tumor size (multifocal) were determined using a receiver operating characteristic (ROC) curve. Next, the area under the curve (AUC), sensitivity, specificity, and positive and negative likelihood ratios were calculated. A p value less than 0.05 was accepted as statistically significant.

RESULTS

We reviewed medical reports from 705 patients diagnosed with PTC between 2010 and 2015. A total of 678 patients were included in the final sample, while 27 patients were excluded due to lost to follow-up. The sample included 564 (83.2%) female and 114 (16.8%) male patients, and the female/male ratio was 4.96. LVI was histologically confirmed in 63/678 (9.3%) patients, while 615/678 (90.7%) patients did not have LVI. Among the female patients, 7.4% had LVI and 18.4% of male patients had LVI. The risk of LVI was 2.80 times greater in the male patients compared to the female patients [95% CI = 1.59-4.95; $p < 0.001$] (Table 1).

The overall mean age was 49.4 ± 12.3 years (min-max: 17-79 years). The mean age for male and female patients was 52.2 ± 14.2 and 48.9 ± 11.8 years, respectively ($p = 0.021$). The mean age of male and female patients with LVI was 44.3 ± 16.7 and 44.6 ± 12.2 years, respectively. The risk of LVI was higher in younger patients compared to older patients [for female patients OR = 0.96; 95% CI = 0.94-0.99; $p = 0.018$; for male patients OR = 0.95; 95% CI; $p = 0.019$] (Table 1 and Figure 1). When the patients were classified according to the age into two groups (i.e. <45 and >45 years old), the ratio of LVI (+) patients was statistically higher in the <45 years group (OR = 2.20; 95% CI = 1.30-3.72; $p = 0.004$). A detailed classification of the patients according to the age decades in relation to the risk of LVI is shown in Table 2. The patients in 17-25 years group had the highest rate of LVI compared to the other age groups [$p = 0.004$] (Table 1).

Preoperative neck US revealed pathological lymph nodes in 37/675 patients. The patients with pathological lymph nodes, preoperatively detected with US, had a statistically higher rate of LVI compared to patients without pathological lymph nodes (OR = 16.69; 95% CI = 8.14-34.24; $p < 0.001$).

The mean tumor size for unifocal tumors was 10.91 ± 12.03 mm (min-max: 0.1-90 mm). The mean tumor size for multifocal tumors was 16.14 ± 15.16 mm (min-max: 0.1-93 mm). The mean unifocal tumor size for patients with LVI was 22.2 ± 14.7 mm and 9.8 ± 11.1 mm in LVI (-) group ($p < 0.001$). The mean tumor size for multifocal tumors was 28.62 ± 20.3 mm in LVI (+) and 14.82 ± 13.9 mm in LVI (-) group ($p < 0.001$). The risk of LVI for every 1 mm of the tumor was 1.05 times greater for unifocal tumors (OR = 1.05; 95%

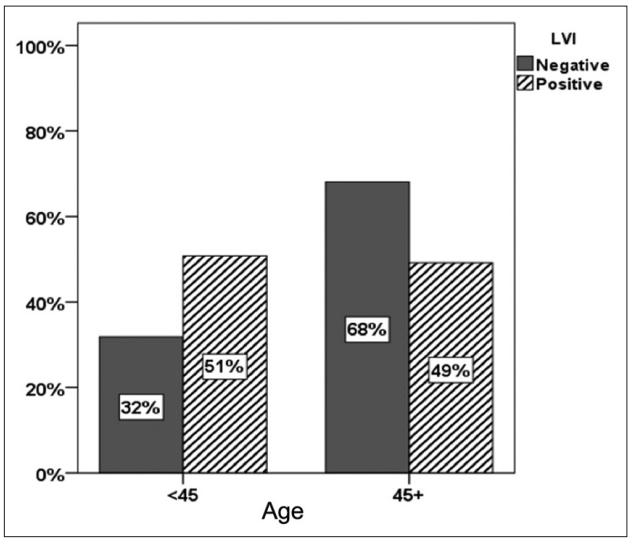

FIGURE 1. A significantly higher risk of lymphovascular invasion (LVI) was observed in younger papillary thyroid carcinoma patients (<45 years old) compared to the patients with >45 years (OR = 2.20; 95% CI = 1.30-3.72; $p = 0.004$).

TABLE 1. Relationship between demographic data and risk of LVI in PTC patients

Parameters	Values	LVI (+)	LVI (-)	*p*	OR (95% CI)
Gender	Female (n=564)	42 (7.4)	522 (92.6)	<0.001	1
	Male (n=114)	21 (18.4)	93 (81.6)		2.80 (1.59-4.95)
Age (years)	Female (n=564)	44.6±12.2	49.2±11.7	0.018	0.96 (0.94-0.99)
	Male (n=114)	44.3±16.7	54.0±13.0	0.019	0.95 (0.92-0.98)
Age (<45 or≥45 years)	<45 (n=228)	32 (14.0)	196 (86.0)	0.004	2.20 (1.30-3.72)
	≥45 (n=450)	31 (6.9)	419 (93.1)		1
Age groups	17-25 (n=19)	6 (31.6)	13 (68.4)	0.004	1
	26-35 (n=78)	11 (14.1)	67 (85.9)	0.080	0.35 (0.11-1.13)
	36-45 (n=147)	16 (10.9)	131 (89.1)	0.018	0.26 (0.08-0.79)
	46-55 (n=212)	13 (6.1)	199 (93.9)	0.001	0.14 (0.04-0.43)
	56-65 (n=153)	11 (7.2)	142 (92.8)	0.002	0.16 (0.05-0.52)
	66+ (n=69)	6 (8.7)	63 (91.3)	0.016	0.20 (0.05-0.74)
Preoperative neck US	Negative (n=638)	42 (6.6)	596 (93.4)	<0.001	1
	Positive (n=37)	20 (54.1)	17 (45.9)		16.69 (8.14-34.24)

Data are presented as mean±standard deviation (SD) and numbers and percentages. OR: Odds ratio; CI: Confidence interval; LVI: Lymphovascular invasion, US: Ultrasonography; PTC: papillary thyroid carcinoma

CI = 1.02-1.08) and 1.04 times greater for multifocal tumors [OR = 1.04; 95% CI = 1.02-1.05] (Table 3).

To discriminate between LVI (+) and LVI (-) cases, ROC curve analysis was performed. The cut-off point for the size of unifocal tumors was >9.5 mm (92.3% sensitivity, 70.4% specificity, and 84% accuracy), and >13 mm for the size of multifocal tumors [82.5% sensitivity, 61% specificity, and 75% accuracy] (Table 4, Figure 2).

Ninety-three patients out of 453 patients were positive for BRAFV600E mutation [BRAF(+) patients]. Among the BRAF(+) patients, 23 had LVI and 70 did not have LVI ($p < 0.001$). The frequency of BRAFV600E mutation was 4.40 times higher in patients with LVI compared to the patients without LVI (95% CI = 2.36-8.20) (Table 5).

Perineural invasion was observed in 12/677 patients (1.7%). Among these patients, perineural invasion was observed in 6/62 patients with LVI (9.7%) and in 6/615 patients without LVI [1%] ($p < 0.001$). The frequency of perineural invasion was 10.87 times higher in patients with LVI compared to the patients without LVI (95% CI = 3.39-34.83) (Table 5).

The number of patients with capsular invasion was 217/678 patients (32%). The risk of capsular invasion was 11.49 times greater in patients with LVI compared to the patients without LVI (OR = 11.49; 95% CI = 5.98-22.09; $p < 0.001$) (Table 5).

ETE was observed in 136/678 patients (20.05%). A statistically significant difference was observed in the frequency

TABLE 2. Frequency of LVI in different age groups of PTC patients

Age groups	17-25	26-35	36-45	46-55	56-65	66+
17-25	1	0.094	0.023*	0.002*	0.005*	0.019*
26-35	0.094	1	0.623	0.052	0.145	0.444
36-45	0.023*	0.623	1	0.153	0.360	0.799
46-55	0.002*	0.052	0.153	1	0.851	0.423
56-65	0.005*	0.145	0.360	0.851	1	0.906
66+	0.019*	0.444	0.799	0.423	0.906	1

*Statistically significant result ($p<0.05$). LVI: Lymphovascular invasion; PTC: papillary thyroid carcinoma

TABLE 3. Relationship between tumor size and LVI in PTC patients

Tumor size (mm)	LVI (+)	LVI (-)	*p*	OR (95% CI)
Tumor size (Unifocal)	22.2±14.7	9.8±11.1	<0.001	1.05 (1.02-1.08)
Total tumor size (Multifocal)	28.62±20.3	14.82±13.9	<0.001	1.04 (1.02-1.05)

Data are presented as mean±standard deviation and numbers and percentages. OR: Odds ratio; CI: Confidence interval; LVI: Lymphovascular invasion; PTC: papillary thyroid carcinoma

TABLE 4. Likelihood of LVI in PTC patients in relation to the tumor size

Tumor size (mm)	Cut-off	Sensitivity (%)	Specificity (%)	LR (+)	LR (-)	AUC	*p*
Tumor size (Unifocal)	>9.5	92.3	70.4	3.12	0.11	0.840	<0.001
Total tumor size (Multifocal)	>13	82.5	61.0	2.12	0.29	0.758	<0.001

LR: Likelihood ratio; AUC: Area under the curve; LVI: Lymphovascular invasion; PTC: papillary thyroid carcinoma

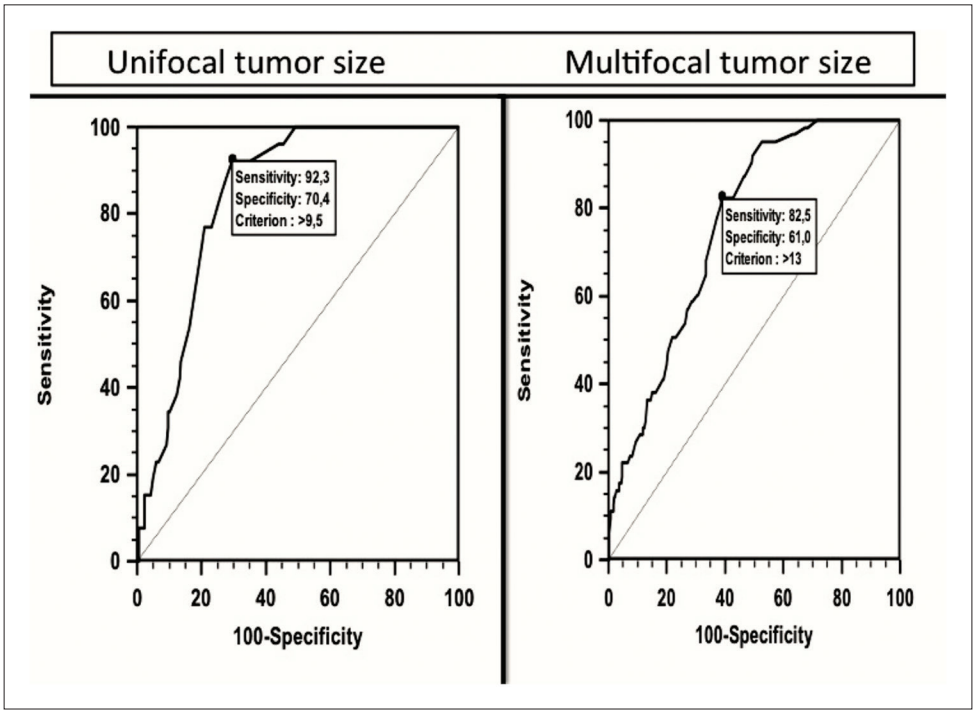

FIGURE 2. To discriminate between lymphovascular invasion [LVI (+)] and LVI (-) cases, receiver operating characteristic (ROC) curve analysis was performed. The cut-off point for the size of unifocal tumors was >9.5 mm (92.3% sensitivity, 70.4% specificity, and 84% accuracy), and >13 mm for the size of multifocal tumors (82.5 % sensitivity, 61 % specificity, and 75 % accuracy).

TABLE 5. Relationship between LVI and histopathological parameters of PTC patients

Parameters	Histopathological outcomes	LVI (+)	LVI (-)	*p*	OR (95% CI)*
BRAF†		n=48	n=405		
	Wild type (n=360)	25 (52.1)	335 (82.7)	<0.001	4.40 (2.36-8.20)
	Mutated (n=93)	23 (47.9)	70 (17.3)		
Perineural invasion†		n=62	n=615		
	Negative (n=665)	56 (90.3)	609 (99.0)	<0.001	10.87 (3.39-34.83)
	Positive (n=12)	6 (9.7)	6 (1.0)		
Tumor capsular invasion†		n=63	n=615		
	Negative (n=461)	12 (19.0)	449 (73.0)	<0.001	11.49 (5.98-22.09)
	Positive (n=217)	51 (81.0)	166 (27.0)		
Extrathyroidal extension†		n=63	n=615		
	Negative (n=542)	15 (23.8)	527 (85.7)	<0.001	19.16 (10.28-35.70)
	Positive (n=136)	48 (76.2)	88 (14.3)		
Lymph node metastasis†		n=53	n=356		
	Negative (n=347)	16 (30.2)	331 (93.0)	<0.001	30.61 (14.99-62.49)
	Positive (n=62)	37 (69.8)	25 (7.0)		
Variant†		n=63	n=615		
	Follicular (n=360)	20 (31.7)	340 (55.3)	<0.001	1‡
	Oncocytic (n=99)	9 (14.3)	90 (14.6)		1.70 (0.74-3.86)
	Classical (n=195)	27 (42.9)	168 (27.3)		2.73 (1.48-5.01)
	Aggressive (n=24)	7 (11.1)	17 (2.8)		7.00 (2.60-18.82)

Data are presented as mean ± standard deviation and numbers and percentages; OR: Odds ratio; CI: Confidence interval; LVI: Lymphovascular invasion
*Odds ratio showed the risk for histopathological outcomes† that was calculated based on LVI (-) as a reference category. ‡Odds ratio showed the risk for PTC variant categories that was calculated based on the follicular variant as a reference category.

of ETE between LVI (+) and LVI (-) group (OR = 19.16; 95% CI = 10.28-35.70; $p < 0.001$) (Table 5).

Lymph node metastasis was observed in 62/409 patients (15.15%). Lymph node metastasis was significantly more common in LVI (+) group compared to LVI (-) group, and the risk was 30.61 times greater in LVI (+) group (OR = 30.61; 95% CI = 14.99-62.49; $p < 0.001$) (Table 5).

Among the 678 patients, only 14 patients (2.1%) had local lymph node recurrences. The frequency of lymph node recurrences was statistically higher in patients with LVI compared to the patients who did not have LVI ($p < 0.001$).

According to the PTC histopathological subtypes, follicular variant was observed in 53.09% (n = 360/678) cases, oncocytic variant in 14.60% (n = 99/678), classic PTC in 28.76% (n = 195/678), and aggressive variants of PTC in 3.53% (n = 24/678) (Table 5). According to the multivariate analysis of the outcomes of histopathological subtypes, the lowest risk of LVI was observed in the follicular variant compared with the other subtypes. The risk of LVI was 1.70 times greater in the patients with oncocytic variant, 2.73 times greater in the classic PTC, and 7.00 times greater in the patients with aggressive variants of PTC.

No significant differences were observed between LVI (+) and LVI (-) groups in terms of the tumor location, presence of multi- or unifocal tumors, number of tumor foci, and the presence of Hashimoto's thyroiditis ($p > 0.05$).

The median follow-up time was 33.54 ± 23.21 months (min-max = 12-160 months). During approximately 3-year median follow-up period, no local lymph node metastasis was observed. Only one patient with distant metastases was detected. Thus, the number of distant metastases was insufficient to conduct a statistical evaluation.

DISCUSSION

In this study, we investigated the correlation between LVI and clinicopathological features of PTC patients. The results showed that the presence of LVI in PTC patients could be considered as a prognostic risk factor of disease worsening, and these patients should be monitored as high-risk PTC patients. The majority of thyroid cancers are PTC [1,27,28]. According to the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute, PTC surveys are based on the stage of cancer in combination with the extension of disease. Although patients with a localized PTC have a 99.9% survival rate, PTC patients with distant metastases have poorer life expectancy (55.3%) [28]. The staging systems for PTC differ between several organizations. EORTC thyroid cancer staging system includes age, gender, tumor differentiation, capsule invasion, and metastasis [6]. Similarly, thyroid cancer staging systems of Mayo Clinic, Lahey Clinic, University of Chicago, and UICC/AJCC are based on the information on age, extension of tumor, gender, and completeness of surgery [6, 29-33]. The definition of LVI in thyroid cancer patients was described by Mete et al. [27] and included the following points: the presence of tumor cells in lymphovascular spaces or endothelium of lymphovascular channels, invasion of tumor cells through a vessel wall and endothelium,

or the presence of thrombus adherent to intravascular tumor. Although LVI is a poor prognostic factor in different cancers, it is not included into the staging systems for PTC [14-20]. A retrospective analysis of breast cancer patients showed that the presence of LVI in these patients indicates a poor prognosis [34,35]. The impact of LVI on the progression and severity of thyroid cancers is discussed only with limited case series, and conflicting results are reported. The frequency of LVI in thyroid cancer ranges from 2% to 14% and it decreases to 5% among PTC patients [9]. In the studies of Gardner et al. [36] and Falvo et al. [37], LVI was observed in 7.5% and 9.5% of PTC patients, respectively. In another study, LVI was determined in 4.9% of PTC patients (n = 662) [38]. The largest study investigating the association between LVI and PTC was published by Pontius et al. [25]. The authors reported 11.6% incidence of LVI in PTC patients [25]. In the current study, we showed 9.3% rate of LVI among the PTC patients.

The association between gender and the severity of PTC is well investigated. The male gender is considered to be a poor prognostic factor in PTC [39]. Several studies in which the association between LVI and gender was examined showed that the rate of LVI in male patients was higher (8.4% to 10.5%) compared to female patients (4.8% to 9.4%) [25,36-38]. We found that the risk for LVI was 2.80 times greater in the male patients compared to the female patients, and this rate (18.4%) was also higher compared to the rates reported in the literature.

Thyroid cancer staging systems also indicate age as a prognostic factor [40]. A number of studies showed that PTC patients older than 45 years have poor prognosis [2,4,6,9,11,12]. However, several studies also indicated worse prognosis and the presenece of lymph node and distant metastasis in younger PTC patients. For example, in a group of 211 PTC patients, Can et al. [29] documented a significantly higher rate of lymph node metastases and LVI in PTC patients younger than 45 years (p = 0.023). In a meta-analysis of Qu et al. [41] patients younger than 45 years had significantly higher rates of nodal metastasis compared to older patients (50.4% versus 37.6%, p = 0.0005). Our results on the association between the age and LVI in the PTC patients mostly do not correspond with the previous studies. In our sample, patients of both genders, generally younger than 45 years, and aged between 17 and 25 years had a significantly higher rate of LVI.

Preoperative US is one of the most important diagnostic tools, and the treatment may be determined based on US results. US findings that indicate malignant characteristics of PTC include ETE, nodal metastasis, and a higher TNM stage compared to PTC with benign characteristics [42,43]. We showed that the PTC patients with suspicious US results for lymph node metastasis had a significantly higher rate of LVI, which is in agreement with previous studies. Furthermore, we suggest that in PTC patients who underwent thyroidectomy and who have LVI and lymph node metastasis, meticulous scanning of lymph nodes should be performed, if they did not have lymph node dissection. On the other hand, in LVI patients without nodal involvement, the follow-up of disease should focus on local neck metastasis.

Tumor size is another parameter for staging PTC. Larger tumors tend to have more lymph node metastasis, ETE, or distant metastasis. Previous studies showed that the tumor size in LVI patients ranged from 1.59-2.8 cm in diameter [25,37,38]. Our study is the first to investigate the cut-off tumor size for unifocal and multifocal tumors. Moreover, we showed, for the first time, that a 1.05 times greater risk of LVI exist for every 1 mm enlargement of the tumor size. The ROC curve analysis showed >9.5 mm for the umor size in unifocal tumors and >13 mm for the tumor size in multifocal tumors. The tumor sizes observed in our study are the smallest tumor sizes reported in the literature that indicate the presence of LVI.

The *BRAF* gene mutation is found in approximately 45% of thyroid cancer patients and most studies indicate that the *BRAF* mutation is a poor prognostic factor in these patients. However, recent studies on the *BRAF* mutation status in PTC patients showed conflicting results; furthermore, limited data exist on the association between the *BRAF* mutation and LVI in PTC patients [44,45]. A recent trial demonstrated that among 60 patients with the BRAF mutation, 90% had LVI. In another study, among 71 BRAF(+) PTC patients, 46% had LVI, and the *BRAF* mutation was found to be a statistically significant risk factor for LVI in these PTC patients [45]. Our study is the largest clinical series investigating the association between the presence of LVI and *BRAF* mutation in PTC patients. The presence of LVI may be an indicator for the presence of *BRAF* mutation in PTC patients, likewise, clinicians may perform a mutation analysis in PTC patients with LVI, to plan the follow-up and treatment.

Capsular invasion, ETE, and perineural invasion are considered to be poor prognostic factors in PTC patients and their coexsistence with LVI was noted in several studies [25,29,36-38,41].

Lymph node metastasis is one of the most important factors affecting preoperative treatment strategies in PTC patients. In the case of lymph node metastasis, the type of surgery, extent of dissection, radioiodine therapy, and the radioiodine dose are adjusted in PTC patients. Lymph node dissection is a controversial issue with regard to the survival benefit. Qu et al. [41] conducted a meta-analysis investigating the association between LVI and lymph node metastasis, and showed that LVI was significantly associated with an increased risk of central lymph node metastasis. Our results showed the higher rate of lymph node metastasis (69%) as well as the higher risk of lymph node metastasis (OR = 30.61) in PTC patients with

LVI. Poor prognostic factors such as capsular invasion, ETE, perineural invasion, and LVI are more frequently observed in the aggressive subtypes of thyroid carcinoma [46]. For example, Mete et al. [26] reported 2.2% rate of LVI in well-differentiated PTC and 26% in poorly differentiated PTC [26].Our results also showed that the highest rate of LVI is associated with the aggressive forms of PTC.

In a retrospective study investigating lymph node recurrence in 210 PTC patients, Conzo et al. [47] showed that locoregional lymph node recurrence is more frequent in young male patients and in primary tumors less than 2 cm in size. In addition, lymph node relapse was more frequently associated with follicular variant PTC [47].

The main limitation of our study are imprecise results on local recurrence of PTC and distant metastasis rates, due to the relatively short follow-up period. The presence of LVI in association with poor prognostic factors of thyroid carcinoma should alert clinicians to closely follow-up and re-evaluate the patient. Even in the case of low-risk patients and lack of histopathological data on lymph nodes, clinicians should monitor the patients with LVI for recurrences. Additionally, LVI might be considered as a parameter in the PTC staging systems, and the presence of LVI may help in determining adjuvant therapy strategies (e.g., radioiodine ablation therapy or L-Thyroxine suppression therapy).

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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