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The association between integrin β4 overexpression and lymphovascular invasion in papillary thyroid cancer

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Abstract

Introduction: Lymphovascular invasion is an independent prognostic marker in papillary thyroid carcinomas. In addition, integrin β4 is associated with advanced progression and metastasis in many malignancies. We aimed to investigate the relationship between integrin β4 and lymphovascular invasion in papillary thyroid carcinoma.

Material and methods: 73 patients with papillary thyroid cancer (48 patients with lymphovascular invasion and 25 patients without) were included in our study. The immunohistochemical staining score for integrin β4 was evaluated according to the percentage and intensity of staining. The staining intensity was scored as 0 (no staining), 1 (weak staining — light yellow), 2 (medium staining — yellow-brown), and 3 (strong staining — brown). The staining was scored by multiplying the percentage and intensity of staining.

Results: The mean percentage of integrin β4 staining was 63.54 ± 22.26% in the group with lymphovascular invasion and 10.2 ± 22.48% in the group without lymphovascular invasion ($p < 0.001$). When evaluated in terms of staining score, it was found to be 107.08 ± 45.29 in the group with lymphovascular invasion and 16.2 ± 40.03 in the group without lymphovascular invasion ($p < 0.001$). There was a linear relationship between the percentage of integrin β4 and the staining scores ($r^2 = 0.881$; $p < 0.001$). In the by receiver-operating characteristic (ROC) curve analysis for the cut-off value of the percentage of integrin β4 staining, the area under the curve was found to be 0.916. The cut-off value for the percentage of integrin β4 was found to be 35 (sensitivity 91.7% and specificity 88%) (odds 80.66%).

Conclusions: A significant relationship was found between integrin β4 expression and lymphovascular invasion in papillary thyroid carcinomas. Integrin β4 expression level can be used as a marker to predict the presence of lymphovascular invasion in papillary thyroid carcinomas, especially in large tumours where it may not be possible to sample the entire tumour. **(Endokrynol Pol 2024; 75 (3): 310–316)**

Key words: *thyroid cancer; integrin β4; lymphovascular invasion*

Introduction

Thyroid cancers are the most common endocrine malignancies, and the most common form is papillary thyroid cancer (PTC). In recent years, an increase in the incidence of PTC from 4.8 to 14.9 per 100,000 has been observed [1]. Although the 5-year survival rate is around 95%, approximately 5–20% of patients have recurrence, and 10–15% have distant metastases [2, 3]. Factors affecting the prognosis of thyroid carcinomas are age, radiation exposure to the head or neck region, tumour size, histopathological subtype, lymph node invasion, and distant metastases [4].

Although lymphovascular invasion (LVI) is a significant predictor of prognosis in many malignancies (such as head and neck squamous cell carcinoma, breast cancer, colorectal cancer, and urothelial cancer), data on the relationship between LVI and thyroid cancers are limited [5]. In some studies on this subject, a relationship was found between the presence of LVI and central and cervical lymph node metastases, extrathyroidal spread, increased recurrence rates, distant metastases, aggressive histological subtypes, tumour size, and age [5–11]. In a study involving a large national dataset, LVI was independently associated with compromised patient survival [6]. Although LVI is not currently a part of any staging system for differentiated thyroid malignancies, it is among the factors that predict recurrence in the risk stratification of the American Thyroid Association (ATA) guideline [4].

Integrins are heterodimers composed of alpha and beta subunits which are transmembrane glycoprotein receptors. Integrin β4 is a member of the integrin family and is expressed on the basement membrane of epithelial cells. It forms a heterodimer with an α6 subunit. α6β4 acts as the main receptor for laminin,

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a basement membrane component. Integrin β4 forms hemidesmosomes with extracellular laminin and intracellular filaments. In the tumoural microenvironment, integrin β4 detaches from hemidesmosomes and moves to actin filaments, thus allowing tumour cells to migrate. It may also act as a signal transducer by activating certain pathways and may increase the progression of many malignancies [12, 13]. In some studies on pancreatic, breast, and bladder cancer, high-level integrin β4 expression has been associated with more aggressive tumour characteristics [14–16]. However, studies evaluating tumour aggressiveness and integrin β4 overexpression in PTCs are limited. Therefore, we aimed to investigate the relationship between integrin β4 and LVI in patients with PTC.

Material and methods

This study was designed as a case-control study to evaluate the data of PTC patients diagnosed and followed in our Endocrinology and Metabolic Diseases Clinic. Local Ethics Committee approval was obtained before the study (TUTF-BAEK 2020/331).

The effect size was calculated as 0.329 based on the study performed by Li et al. [12]. Considering the calculated effect size, it was decided to include a total of 73 patients (48 patients with LVI and 25 patients without LVI) in the study with a 5% significance level and 80% power. Seventy-three patients who met the inclusion criteria determined for the study by scanning from the information operating system of our hospital were selected by simple random sampling. Patients with incomplete data and those who followed up with any other malignancy were not included in the study. A completed informed Voluntary Consent Form was obtained from each of the patients included in the study. Gender, age at the time of diagnosis, tumour size, lymph node metastasis, histopathological variants, staging at the time of diagnosis, and presence/absence of LVI were examined as clinical and demographic characteristics. Solid, tall cell, and hobnail variants were considered as aggressive variants, while classical, follicular, and oncocytic variants were included in the non-aggressive variants. The subjects were staged according to the 8th Edition of the American Joint Committee (AJCC) Cancer Staging Manuel [17].

Histopathological evaluation

Haematoxylin-eosin stained slides of the subjects were re-evaluated simultaneously by 2 pathologists using a Nikon Eclipse Ci-L model microscope, and slides containing tumours in sufficient quantity and quality were included in the study. Paraffin blocks were extracted from the laboratory archive of the Medical Pathology Department. For immunohistochemical examination, 5-micron sections of paraffin blocks were prepared on positively charged slides with a Leica rotary microtome. Cell-signalling rabbit monoclonal antibody at 1/200 dilution was used for integrin β4 immunohistochemical staining. The Optiview Universal DAB Detection Kit was used in the Ventana Medical System-Benchmark XT/ISH Staining module. Breast tumour tissue was chosen as positive control tissue. The staining score for integrin β4 was evaluated according to staining percentage and intensity. Staining intensity was scored as 0 (no staining), 1 (weak staining — light yellow), 2 (medium staining — yellow-brown), and 3 (strong staining — brown) (Fig. 1). A definite positive reaction was defined as a brown signal, a reaction of integrin β4 with the peripheral nerve. In addition, the percentage of staining in existing tumor cells was evaluated out of 100. Those with a staining percentage of 33% and above were evaluated as having high level of integrin β4 expression [12]. The staining score was obtained by multiplying the staining percentage and intensity. In this criterion, which was evaluated out of 300, 66, and above (33% × 2), was expressed as high staining and below 66 as low staining.

Statistical evaluation

The data were statistically evaluated using the SPSS (Statistical Package for the Social Sciences Version 21.0; SPSS Inc. Chicago, IL, Unit-

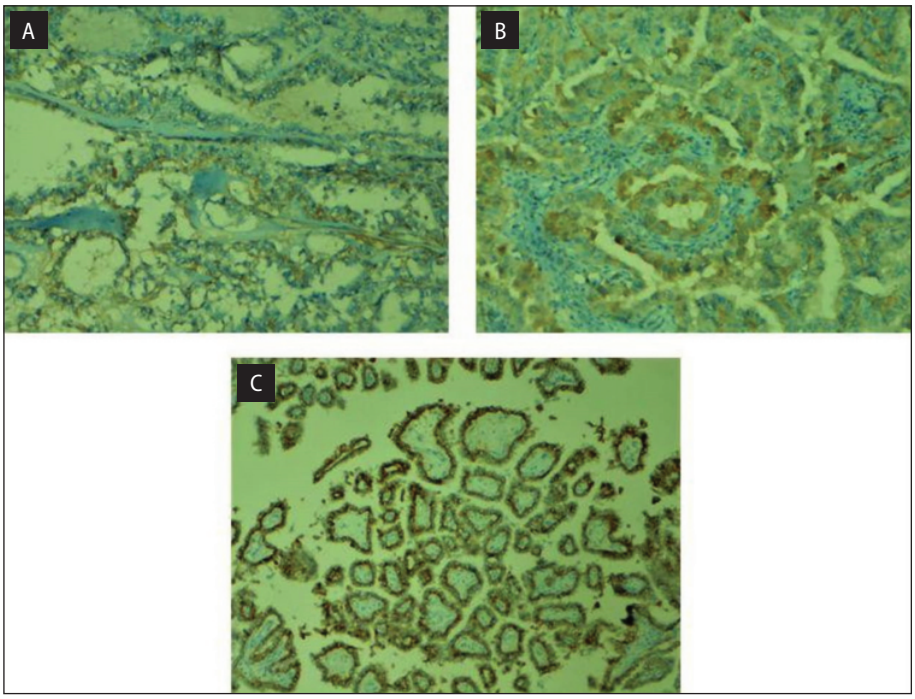


Figure 1. *Integrin β4 staining intensity.* **A.** *1+ Staining;* **B.** *2+ Staining;* **C.** *3+ Staining (×200)*

ed States) program. The assumption of normal distribution for continuous variables was determined with the Kolmogorov-Smirnov Test. Continuous parameters with kurtosis and skewness values between ± 1.5 were considered to be normally distributed [18]. Continuous variables are expressed as the median (interquartile range) or mean (± standard deviation [SD]) and compared by Student's t-test or the Mann-Whitney U-test as appropriate. Categorical variables were stated as number (n) and percentage (%). The relationship between continuous data was examined by Pearson correlation analysis. Comparisons of categorical variables were assessed by the chi-square test or Fisher's exact test. The discriminative capacity of integrin β4 expression was assessed by receiver-operating characteristic (ROC) curves and their area under the ROC curve (AUC). Optimal cut-off values of integrin β4 expression were determined by Youden's index. The logistic regression model was used to determine the independent relationship between LVI and other parameters. $P < 0.05$ was considered as statistically significant.

Results

The patients included in the study were between 18 and 65 years old, and the mean age was 42.51 (±12.58) years. Forty (54.8%) of the patients were female, and 33 (45.2%) were male. LVI was detected histopathologically in 48 patients, and LVI was not present in 25 patients. Both the percentage and the intensity score of integrin β4 staining were significantly higher in the group with LVI than in those without. In addition, patients with a staining score of integrin β4 ≥ 66 had a statistically significant higher incidence of LVI. In addition, the incidence of lymph node metastasis was higher in the patient group with LVI. Demographic, clinical, and histopathological features between the 2 groups with and without LVI are shown in Table 1.

A statistically significant correlation was observed between the percentage and intensity levels of integrin β4 (Chi-square = 0.881; $p < 0.001$). Variables that showed a significant difference in terms of LVI in simple statistical analyses were included in the logistic regression analysis. The results are reported in Table 2. Due to the collinearity between the percentage and intensity of integrin β4 staining, the intensity scores were removed in the 2nd step of the backward method. A statistically significant relationship was found between the percentage of integrin β4 and LVI ($p < 0.001$).

Table 1. *Demographic, clinical, and histopathological features according to the presence of lymphovascular invasion*

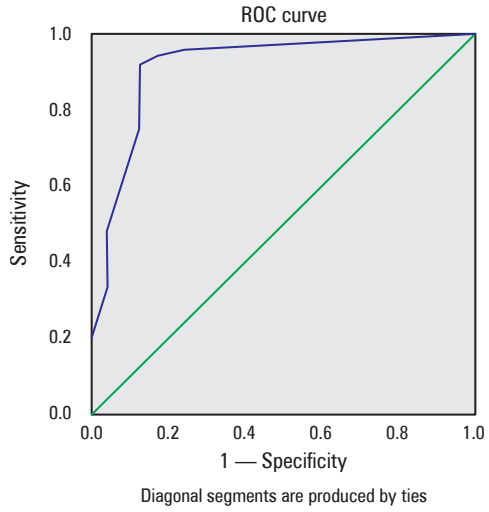
	Lymphovascular invasion (+) (n. %)	Lymphovascular invasion (–) (n. %)	p
Age (years)	40.44 ± 12.60	46.48 ± 11.80	0.510[a]
Sex (Female/Male)	25 (52.1 %)/23 (47.9%)	15 (60%)/10 (40%)	0.519[b]
Histologic variant			
Aggressive	21 (43.8%)	8 (32%)	0.330[b]
Non-aggressive	27 (56.3%)	17 (68%)	
Tumour focality			
Unifocal	15 (31.3%)	12 (48%)	0.160[b]
Multifocal	33 (68.8%)	13 (52%)	
Lymph node metastasis			< 0.001[b]
Yes	30 (62.5%)	1 (4%)	
No	18 (37.5%)	24 (96%)	
Tumour size			
≤ 1 cm	8 (17%)	2 (8%)	0.478[c]
> 1 cm	40 (83%)	23 (92%)	
Tumour stage (AJCC 8th)			
I	45 (93.8%)	25 (100%)	0.698[c]
II	2 (4.2%)	0	
III	1 (2.1%)	0	
Percentage of integrin β4 staining (%) (Mean ±SD)	63.54 ± 22.26	10.20 ± 22.48	< 0.001[a]
Staining score of integrin β4 (Mean ±SD)	107.08 ± 45.29	16.20 ± 40.03	< 0.001[a]
Staining score of integrin β4			< 0.001[b]
< 66	2 (8.0%)	42 (87.5%)	
≥ 66	23 (92.0%)	6 (12.5%)	

n — number of patients; AJCC — American Joint Committee on Cancer; [a]independent samples T-test; [b]Pearson's chi-square; [c]Fisher's Exact Test; SD — standard deviation

Table 2. *Logistic regression analysis findings*

	Model 1				Model 2			
	SE	p	Exp (β)	95% CI	SE	p	Exp (B)	95% CI
Percentage of integrin β4 staining (%)	0.03	0.096	1.052	0.991–1.116	0.014	< 0.001	1.073	1.004–1.103
Staining score of integrin β4	0.17	0.484	1.012	0.979–1.046	–	–	–	–

SE — standard error; Exp(β) — exponential β; CI — confidence interval



Area Under the Curve

Area	Stdandard error	Asymptotic Sig.	Asymptotic 95% CI Lower bound	Asymptotic 95% CI Upper bound
0.916	0.039	0.000	0.839	0.992

Figure 2. *Receiver-operating characteristic (ROC) curve analysis for the optimal value of percentage of integrin β4 staining. CI — confidence interval*

Table 3. *Integrin β4 staining according to lymph node metastasis status*

	Lymph node metastasis (+)	Lymph node metastasis (–)	p
Percentage of integrin β4 staining (%) (Mean ± SD)	64.19 ± 25.00	31.31 ± 32.82	< 0.001[a]
Staining score of integrin β4 (Mean ± SD)	107.10 ± 47.27	52.98 ± 60.76	< 0.001[a]

[a]Independent samples T-test; SD — standard deviation

ROC curve analysis was performed to determine the optimal cut-off value of the percentage of integrin β4 between the presence and absence of LVI (Fig. 2). The cut-off value of the percentage of integrin β4 staining was found to be 35%, with a sensitivity of 91.7% and a specificity of 88% (odds ratio 80.66%).

Additionally, a statistically significant difference was found in both the percentage and intensity score of integrin β4 staining in the 2 groups with and without lymph node metastasis (Tab. 3).

Discussion

Our study investigated the relationship between integrin β4 expression and LVI in patients with PTC. We found that integrin β4 expression was significantly higher in the patient group with LVI.

The incidence of LVI in patients with PTC was found to be between 2 and 10% [19]. LVI is predicted to be an independent risk factor among prognostic markers. In their study with the data of 45415 PTC patients (5284 of them have LVI), Pontius et al. [6] found aggressive features such as cervical lymph node and distant metastases, extrathyroidal spread, aggressive histopathological subtypes, and multifocality to be more common in the group with LVI. In addition, they found that the 5-year survival in the group over the age of 45 years was higher in patients without LVI than in patients with LVI [6]. Two different studies have shown that the presence of LVI is associated with an increased risk

of recurrence [5, 7]. Several other studies have demonstrated a positive association between the presence of LVI and central or cervical lymph node metastases [5, 7, 9–11]. In our study, the rate of lymph node metastasis was observed to be higher in individuals with LVI compared to those without LVI, and this finding was also statistically significant ($p < 0.001$). The results of the studies mentioned above suggest that LVI is a significant risk factor, although it is not currently included in the guidelines as a prognostic risk factor. These studies also show that more attention should be paid to the development of lymph node metastasis in the clinical follow-up of patients with LVI.

It has been determined that integrins play important roles in signalling pathways that enable tumour progression [20, 21]. There are 24 known mammalian integrins. Among them, integrin β4 (α6β4 integrin) is a 1017 amino acid long protein with cytoskeletal and signalling functions [22]. Integrin β4 is thought to act on tumourigenesis and angiogenesis by activating signals necessary for the regulation of the cells' survival, proliferation, and migration properties [23]. Some studies have shown that integrin β4 improves the signalling function of multiple oncogenic receptor tyrosine kinases such as Met, EGF-R, and ErbB2 [24–26]. In a genetic study, Nikolopoulos et al. [27] showed that integrin β4 is expressed in tumour blood vessels and enhances tumour angiogenesis. In studies involving patients with solid tumours, high integrin β4 expression levels have been associated with more aggressive tumour features [14–16]. In the study by Sharifi et al. [28], in which the effect of integrin β4 expression on patients with metastatic breast cancer was investigated, circulating tumour cells were examined. Cells expressing integrin β4 were detected in 61% of the samples taken and were thought to be associated with a poor prognosis. Additionally, Ruan et al. [29], in their study on 2 different mouse models, showed that integrin β4-targeted immunotherapies inhibited tumour growth and lung metastases in breast and head and neck squamous cell tumours.

Studies investigating the relationship between integrin β4 and poor prognostic factors in PTCs are limited. First, Lohi et al. [30] suggested that the interaction between integrin β4 and laminin-5 may activate cell proliferation in thyroid carcinoma. Additionally, Serini et al. [31] found that integrin β4 neo expression was observed in clinically and histologically aggressive thyroid carcinomas. In subsequent years, in the study of Li et al. [12], the expression levels of integrin β4 in patients with PTC were investigated. After the histopathological blocks were stained immunohistochemically, the staining percentages were evaluated. Integrin β4 expression levels were examined in 30 patients with LVI and 93 patients without LVI, and it was found that integrin β4 was expressed statistically significantly more in the group with LVI. The same study found a statistically significant relationship between integrin β4 overexpression and extrathyroidal spread, tumour size, lymph node metastasis, and TNM staging. In our study, integrin β4 expressions were investigated in a total of 73 patients, 48 with LVI and 25 without LVI. After 73 slides were stained immunohistochemically, the percentages of integrin β4 staining and, as a second parameter, their intensity scores were examined. Similarly to the study of Li et al. [12], a positive and significant relationship was found between LVI and both percentages and intensity levels of integrin β4 staining. Li et al. [12] evaluated the staining above 33% as high expression and found higher expression levels in the group with LVI. We obtained a new cut-off value for integrin β4 with 91.7% sensitivity and 88% specificity. This value was calculated as 35%, and a significant difference was found between the groups with and without LVI in terms of integrin β4 staining percentages. In another study conducted on PTC, invasive breast carcinoma, and gastric adenocarcinoma, Li et al. [32] reported that integrin β4 would be more diagnostic than haematoxylin-eosin for lymphovascular and perineural invasion.

In our study, it was determined that integrin β4 expression was also associated with lymph node metastasis. Integrin β4 expression was higher in patients with lymph node metastasis. In a study by Kitajiri et al. [33], a relationship was found between the development of lymph node metastasis and integrin β4 expression in PTC, similarly to our study.

When dealing with large thyroid carcinomas, it may not be possible to sample the entire tumour, which can cause a missed diagnosis of LVI. Sometimes, the presence of LVI in the tumour may be overlooked because it does not correspond to the section taken. In PTC, integrin β4 may show immune expression. In these cases, an increased expression of integrin β4 may serve as a warning to carefully evaluate multiple sections and take samples meticulously to ensure the detection of LVI and lymph node metastasis.

Conclusions

Our study found that there is a significant correlation between the expression of integrin β4 and LVI. Furthermore, patients with lymph node metastasis had higher levels of integrin β4 expression. Data on the relationship between thyroid carcinomas and integrin β4 expression are limited in the literature. While LVI is present in the high-risk category in the PTC risk classification, integrin β4 is not involved in this

category. As more studies on this subject are carried out, integrin β4 might be used as a predictive marker for LVI in PTCs. Cases with high integrin β4 expression can be followed more closely. It can be considered an independent indicator of high risk if the strength of the association is demonstrated in future studies.

Study limitations

The limitation of our study is the limited number of our sample. Future studies with larger samples will yield more effective results. Additionally, the patients' follow-up data were insufficient to evaluate surveillance and recurrence. Therefore, due to the study design, a definitive relationship between integrin β4 and prognosis could not be evaluated. However, finding a relationship between integrin β4 and LVI and lymph node metastasis may pave the way for further and more comprehensive studies related to the prognosis of papillary thyroid carcinoma.

Appendices

This study was presented as an oral presentation at the 43rd Turkish Endocrinology and Metabolic Diseases Congress on 18–22 May 2022.

This article was produced from the internal medicine graduate thesis titled "The association between integrin β4 overexpression and lymphovascular invasion in papillary thyroid cancer" prepared and written by Büşra Tunç in 2022, Trakya University, Faculty of Medicine.

Conflict of interest

The authors declared no conflict of interest.

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Data availability statement

Data available on request from the authors.

Ethics statement

The study was designed in accordance with the principles stated in the Declaration of Helsinki. Local Ethics Committee approval was obtained before the study (TUTF-BAEK 2020/331).

Author contributions

All authors conceived the study design. B.T.T., C.T., and B.A. were involved in data collection. Preparation, staining, and interpretation of pathology slides were performed by C.T., B.T.T., and E.T. B.T.T., S.G., M.C., B.A., B.Y.B., and A.C.Y. performed the statistical analysis. All authors interpreted the data and prepared the manuscript draft. All authors critically reviewed the final version of the manuscript. All authors approved the final version of the manuscript.

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