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The Value of Ki-67 Labeling Index in Central Lymph Node Metastasis and Survival of Papillary Thyroid Carcinoma: Evidence From the Clinical and Molecular Analyses

Yi Lei, MD[1,2,3,†], Xin Zhao, MD[3,4,†], Yang Feng, MD[5], Danshuang He, MD[5], Daixing Hu, MD[5], and Yu Min, MD[5,6]

Abstract

Background: Recent evidence suggests that the Ki-67 labeling index is associated with lymph node metastasis and the prognosis of papillary thyroid carcinoma (PTC).

Methods: We retrospectively evaluated the clinicopathological features of consecutive PTC patients between Jan 2019 and Oct 2020 in our medical center. The molecular analysis was also conducted by using the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) program. The Chi-square test was performed for the comparison of variables between patients with central lymph node metastasis (CLNM) and not. Besides, univariate and stepwise multivariate logistic regression analyses were further used to determine the risk factors for CLNM in PTC.

Results: Our results showed that male gender (odd ratio (OR) = 3.02; 95% CI: 1.81-5.04), tumor size >1 cm (OR = 2.81; 95% CI: 1.84-4.29), multifocality (OR = 2.08; 95% CI: 1.31-3.30, and Ki-67 labeling index (>3% and ≤5%: OR = 1.20; 95% CI: .73-1.97; >5%: OR = 3.85; 95% CI: 1.62-9.14) were independent risk factors for CLNM. After excluding the patients with harvested central lymph nodes <3, increased Ki-67 labeling index was still associated with the number of CLNM and the lymph node ratio. Additionally, the expression level of Ki-67 was significantly correlated with a higher N stage and worse disease-free survival in TCGA and validated GSE60542 datasets.

Conclusions: Higher Ki-67 labeling index (>5%) is significantly associated with the CLNM in PTC patients, like other indicators of the male gender, larger tumor size, and multifocality. Besides, the Ki-67 was also determined to be associated with CLNM and DFS in PTC patients, which may act as an important molecular marker in PTC.

Keywords

papillary thyroid carcinoma, lymph node metastasis, Ki-67, risk factor, the cancer genome atlas

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[1]Department of Endocrinology and Metabolism, The Affiliated Hospital of Southwest Medical University, Luzhou, China
[2]Cardiovascular and Metabolic Diseases Key Laboratory of Luzhou, Luzhou, China
[3]Sichuan Clinical Research Center for Nephropathy
[4]Department of Urology, The Affiliated Hospital of Southwest Medical University, Luzhou, China
[5]Department of Breast and Thyroid Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China
[6]Department of Biotherapy and National Clinical Research Center for Geriatrics, Cancer Center, West China Hospital, Sichuan University, Chengdu, China

[†]These authors have contributed equally to this work and share the first authorship.

Corresponding Author:
Yu Min, MD, Department of Breast and Thyroid Surgery, The Second Affiliated Hospital of Chongqing Medical University, No.74, Linjiang Rd, Yuzhong Dist, Chongqing 404100, China.
Email: 13108175138@163.com

Introduction

Nowadays, thyroid cancer shows a significantly increased prevalence and diagnosis around the world.[1-4] Among different histological variants of thyroid carcinoma, papillary thyroid carcinoma (PTC) is the most frequent subtype and often presents a favorable prognosis.[3,5,6] However, extensive regional lymph node metastasis could reverse this condition. Namely, it impairs long-term disease-free survival (DFS) and could further increase the reoperation rate among these patients.[7,8] Notably, following the latest clinical adult differentiated thyroid carcinoma (DTC) management guideline from the American Thyroid Association (ATA) 2015, lobectomy without central lymph node dissection (CLND) has been considered to be a promising surgical extension for clinically lymph node-negative (cN0) DTC patients in developed countries.[9] But whether this strategy is optimally suitable for patients from developing countries is still controversial.[10] Therefore, how to better manage the central compartment of PTC needs further exploration. For this reason, individualized predicting of the central lymph node metastasis (CLNM) for guiding the surgical extension could not only help decrease the risk of postoperative complications but enhance the benefits of surgical intervention. Recently, compelling evidence has proved that the male gender, younger age (≤55 years), extrathyroidal invasion (EI), large tumor size, and multifocality were significantly associated with regional lymph node metastasis in patients with PTC.[11-14]

Notably, as an important immunohistochemical biomarker, the Ki-67 labeling index was frequently used to evaluate cellular proliferation in the progression of different solid tumors.[15,16] Regarding thyroid cancer with relatively low expression levels of the Ki-67 labeling index, several previous studies have explored the association between expression levels of the Ki-67 labeling index and the clinical features and prognosis of thyroid cancers, especially in terms of PTC.[17-21] However, whether these associations also existed in Chinses population was not well documented.

In the present study, we aim to find the clinical risk factors in CLNM of PTC patients based on our medical center experience. Besides, we also aim to evaluate the role of the Ki-67 labeling index in CLNM of PTC patients and the prognostics in this subpopulation during the postoperative follow-up via analyzing the patients' data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases.

Materials and Methods

Study Population and Data Source

The data of the current study was retrospectively derived from two parts. On the one hand, to identify the clinical risk factors in CLNM of PTC patients and the relation between the Ki-67 labeling index and CLNM, the patient data were collected from our department, a high-volume medical center in Southwest China, between Jan 2019 and Oct 2020. On the other hand, to evaluate the role of the Ki-67 labeling index in the prognostics of PTC patients, patient data was collected via gene expression data and survival information from TCGA (https://tcga-data.nci.nih.gov/tcga/) and GEO (https://www.ncbi.nlm.nih.gov/geo/) databases. The data analysis of TCGA was based on GEPIA (http://gepia.cancer-pku.cn/), a web-based bioinformatic analysis tool. Immunohistochemical expression of the Ki-67 labeling index in thyroid cancer tissues and normal tissues was obtained from the Human Protein Atlas (https://www.proteinatlas.org). As the molecular and genetic data were extracted from the TCGA and GEO databases, they did not contain any personally identifiable information. Informed consent and ethical approval were not required. The process of patient selection was showed in Figure 1. The study was conducted following statements of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[22]

Immunohistochemistry for Ki-67 Labeling Index Evaluation

In our hospital, the specimen of the thyroid was managed following a standardized process. First, thyroid tissues were macroscopically evaluated immediately after the excision from the patients and then fixated in formalin (10% concentration, PH7.2-7.4). Then, the paraffin blocks with the dehydrated specimens were melted and the specimens were placed on metal molds. Molten paraffin wax at 60°C was poured into a cassette for re-fixation, followed by a cooling period. Later on, the specimens were cut with a 4 μm thickness for further histological investigations. The immunohistochemical staining for calculating the Ki-67 expression in the representative section of the primary tumor by using a routine standardized methodology at our center. And the Ki-67 labeling index is calculated by manual counting of the amount of Ki-67 positive cells (only nuclear staining) divided by the total amount of tumor cells in "hot-spot" regions, counting at least 2000 cells.

Variable Definition and Evaluation

The following clinicopathological characteristics were collected and analyzed to establish a retrospective database: gender (male and female), age (between 18 and 80 years; divided into 2 groups according to the 8th edition of the AJCC TNM staging system: <55 years and ≥55 years), $BRAF^{V600E}$ mutation status (mutant, wild-type, or unknown), Ki-67 labeling index (Owing to the low expression levels of Ki-67 labeling index in PTC and most of the patients were at levels of 0 or 1% Ki-67 labeling index in our study, it was analyzed as categorical variable according to previous studies[18-20] on this topic: ≥0% and ≤3%, >3% and ≤5%, >5%), primary tumor size (the largest diameter ≤1 cm and >1 cm), and multifocality (defined as more than 2 primary tumor focus in the thyroid).

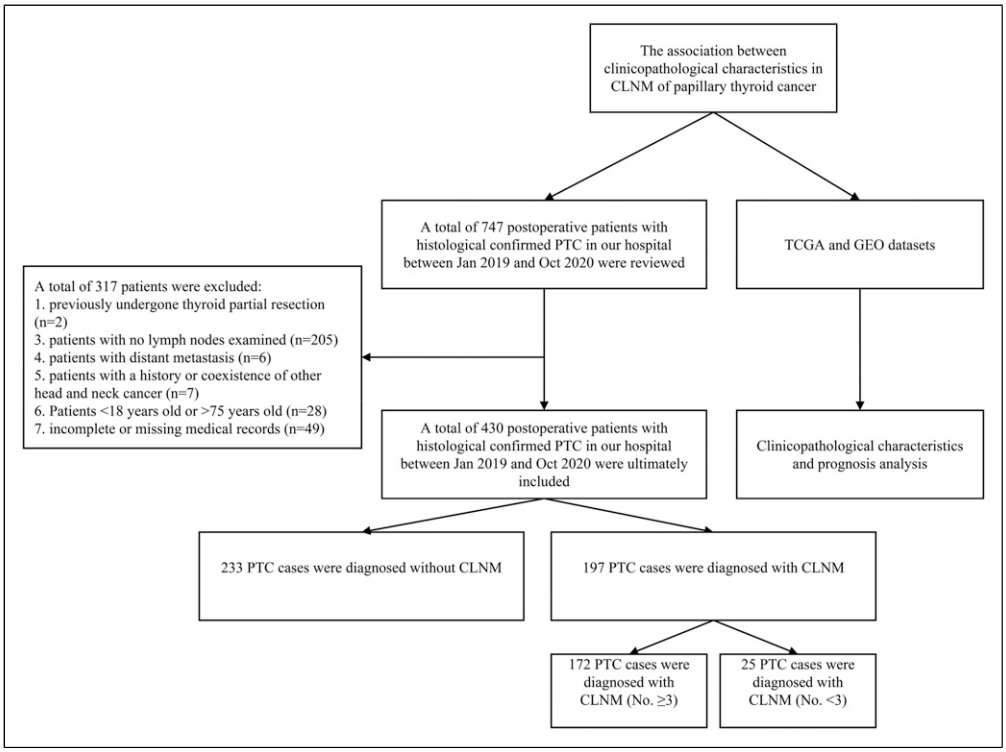

Figure 1. The patient selection process for this study.

Surgical Information: All operations were performed by experienced thyroid surgeons in our department and all thyroid tissues and regional harvested lymph nodes were evaluated and confirmed by two senior pathologists from the Department of Pathology in our medical center. The surgical method (conventional thyroidectomy and endoscopic thyroidectomy were performed), surgical extension (lobectomy, subtotal thyroidectomy, total thyroidectomy, and radical thyroidectomy), regional lymph node harvested, and regional lymph node-positive (The diagnosis of CLNM was based on the findings of typical cancer cells or atypical thyroid tissue involved in the lymph node after the hematoxylin-eosin staining) were calculated. The thyroid function including thyroid stimulating hormone (TSH, Non-pregnancy reference: .35-5.00 μIU/ml), thyroglobulin antibody (TgAb, reference: .00-115.00 IU/mL), thyroid peroxidase antibody (TPOAb, reference: .00-34.00 IU/mL), thyroglobulin (Tg, reference: 1.40-78.00 μg/L) were recorded.

Statistical Analysis

The categorical variables in PTC patients were compared using the Pearson-chi square test or Fisher's exact chi-square test (based on the expected value), The continuous variables were compared using Student's two-tailed t-test. Univariate logistic regression analysis was initially used to identify the potential risk factors involved in CLNM of PTC patients. Moreover, only statistically significant variables were included for stepwise multivariate analysis. The missing data were addressed by filling up the mean value of the group. A *P*-value of <.05 was defined as the necessary criterion for variables when performing further multivariate analyses. The recurrence-free survival analysis was assessed by Kaplan–Meier curves to compare the high and low expression of Ki-67. All analyses were conducted by using the SPSS 25 (SPSS/IBM, Chicago, IL, USA) and R 3.3.2 software.

Results

Clinical Characteristics of Papillary Thyroid Carcinoma Patients

Generally, a total of 430 PTC patients from our hospital were ultimately enrolled in the present study. Among them, 197 cases (45.8%) confirmed the CLNM, and approximately 82% of patients (162/197) were under the age of 55 years old at diagnosis. A high frequency of the $BRAF^{V600E}$ mutation was observed in patients who received the $BRAF^{V600E}$ mutation test, with a rate of 94.2% (340/361 cases). However, it did not reach a significant difference between patients with CLNM and patients without CLNM (P = .928). Although females counted for the majority of CLNM patients (138 cases vs 59 cases), male patients had a higher risk of CLNM (P < .001). Besides, the tumor size (P < .001) and multifocality (P < .001) were remarkably different in PTC patients concurrent with CLNM or not. Notably, the CLNM rate increased with the increase of the Ki-67 proliferation index (P = .014). Additionally, the mean serum level of TgAb in patients with

CLNM was much higher than in patients without CLNM (306.70 ± 739.80 IU/mL vs 165.78 ± 365.96 IU/mL, $P = .044$). Meanwhile, no significant difference was observed between the mean serum level of TPOAb in patients with CLNM and patients without CLNM (71.32 ± 115.31 IU/mL vs 85.89 ± 143.37 IU/mL, $P = .112$). The detailed clinical characteristics of PTC patients in the study were summarized in Table 1.

Univariate and Multivariate Logistic Regression Analyses

The univariate analysis revealed that male gender (odd ratio (OR) = 2.68; 95% confidence intervals (CI): 1.66-4.35, $P < .001$), tumor size >1 cm (OR = 2.90; 95% CI: 1.94-4.33, $P < .001$), multifocality (OR = 2.28; 95% CI: 1.48-3.51, $P < .001$), and Ki-67 labeling index (>3% and ≤5%: OR = 1.36; 95% CI: .86-2.16; >5%: OR = 3.03; 95% CI: 1.34-6.88; $P = .018$) were significantly associated with the CLNM in PTC patients. However, there was no significant correlation with CLNM among PTC patients, as referring to the age at diagnosis ($P = .396$), B-raf^{V600E} status ($P = .915$), TSH level ($P = .110$), TgAb ($P = .251$), TPOAb ($P = .200$), and Tg ($P = .689$) (Table 2). The multivariate logistic regression forest plot showed that male gender (OR = 3.02; 95% CI: 1.81-5.04; $P < .001$), tumor size >1 cm (OR = 2.81; 95% CI: 1.84-4.29; $P < .001$), multifocality (OR = 2.08; 95% CI: 1.31-3.30; $P = .002$), and expression levels of Ki-67 labeling index (>3% and ≤5%: OR = 1.20; 95% CI: .73-1.967; >5%: OR = 3.85; 95% CI: 1.62-9.14; $P = .009$) were the independent risk factors in CLNM (Figure 2).

Ki-67 Expression Level in Papillary Thyroid Carcinoma Patients and Its Association With the Survival

Among 172 PTC patients with coexistence CLNM with harvested number of central lymph nodes no less than 3, there was a slightly significant correlation between the Ki-67 labeling index and the number of positive central lymph nodes, however, there was no statistical significance (Table 3). Also, the burden of CLNM (defined as positive central lymph nodes/harvested number of central lymph nodes, excluding patients with one or two harvested number of central lymph nodes) was more serious in patients with high expression levels of Ki-67 (Table 3), especially in terms of the comparison in the group (Ki-67 labeling index:>3% and ≤5%) and group (Ki-67 labeling index:>5%, $P = .016$).

Reviewing the TCGA database, the expression levels of Ki-67, as one of the pivotal important proliferation markers in multiple solid tumors' progression (Figure 3A), were confirmed significantly higher in PTC patients when

Table 1. Clinicopathological Features of 430 PTC Patients With (Without CLNM) in the Present Study.

Variables	Subgroup	No. (%) of Patients		
		With CLNM (n = 197)	Without CLNM (n = 233)	*P*
Gender	Male	59 (30)	32 (14)	[a]**<.001**
	Female	138 (70)	201 (86)	
Age	<55	162 (82)	184 (79)	[a]0.395
	≥55	35 (18)	49 (21)	
Size	≤1 cm	95 (48)	170 (73)	[a]**<.001**
	>1 cm	102 (52)	63 (27)	
BRAFV600 E mutation	No	10 (5)	11 (5)	[b]0.928
	Yes	154 (78)	186 (80)	
	N/A	33 (17)	36 (15)	
Multifocality	No	125 (63)	186 (80)	[a]**<.001**
	Yes	72 (36)	47 (20)	
Surgical method	Conventional	114 (58)	113 (48)	/
	Endoscopy	83 (42)	120 (52)	
Surgical extension	Lobectomy	38 (19)	84 (36)	/
	Subtotal thyroidectomy	9 (5)	10 (4)	
	Total thyroidectomy	134 (68)	139 (60)	
	Radical thyroidectomy	16 (8)	0 (0)	
Ki-67	≥0% and ≤3%	129 (66)	176 (75)	[b]**0.014**
	>3% and ≤5%	48 (24)	48 (21)	
	>5%	20 (10)	9 (4)	
TSH	—	*3.49 ± 9.13	*2.81 ± 5.50	[c]0.346
TgAb	—	*306.70 ± 739.80	*165.78 ± 365.96	[c]**0.044**
TPOAb	—	*71.32 ± 115.31	*85.89 ± 143.37	[c]0.112
TG	—	*46.49 ± 97.34	*40.31 ± 93.00	[c]0.447
CLN examined	—	*6.95 ± 4.34	*4.25 ± 3.57	[c]**<.001**

Abbreviation: PTC: papillary thyroid carcinoma; CLNM: central lymph node metastasis; TSH: thyrotropin; TgAb: anti-thyroglobulin antibody; TPOAb: anti-thyroid peroxidase antibody; TG: thyroglobulin; CLN: central lymph node.
Bold values indicate statistical significance ($P < .05$).
*Mean ± SD.
[b]Two-tail Fisher exact test.
[a]Pearson's Chi-squared test.
[c]Student's two-tail t-test.

Table 2. Univariate logistic regression analysis of 430 PTC patients for CLNM.

		Univariable	
Variables	Subgroup	Odd Ratio	*P*
Gender	Female	Reference	**<.001**
	Male	2.68 (1.66-4.35)	
Age	<55	Reference	.396
	≥55	.81 (.50-1.31)	
Tumor size (cm)	≤1	Reference	**<.001**
	>1	2.90 (1.94-4.33)	
Multifocality	No	Reference	**<.001**
	Yes	2.28 (1.48-3.51)	
B-raf^{V600E} mutation	No	Reference	.915
	Yes	.91 (.38-2.20)	
	N/A	1.01 (.38-2.68)	
Ki-67	≥0% and ≤3%	Reference	**.018**
	>3% and ≤5%	1.36 (.86-2.16)	
	>5%	3.03 (1.34-6.88)	
TSH (μIU/ml)	Normal	Reference	.110
	High	1.85 (.87-3.95)	
TgAb (IU/ml)	Normal	Reference	.251
	>115 and ≤575	.92 (.56-1.53)	
	>575 and ≤1150	.80 (.32-2.02)	
	>1150	2.61 (.97-7.03)	
TPOAb	Normal	Reference	.200
	>34 and ≤170	.75 (.44-1.25)	
	>170 and ≤340	1.15 (.60-2.17)	
	>340	.41 (.16-1.08)	
TG (μg/L)	≤78	Reference	.689
	>78	1.14 (.60-2.17)	

Abbreviation: PTC: papillary thyroid carcinoma; CLNM: central lymph node metastasis; TSH: thyrotropin; TgAb: anti-thyroglobulin antibody; TPOAb: anti-thyroid peroxidase antibody; TG: thyroglobulin.
Bold values indicate statistical significance ($P < .05$).

compared with normal thyroid tissues (Figure 3B, $P < .001$). Additionally, a significantly higher Ki-67 mRNA expression level was observed in lymph node-positive patients, compared with negative lymph node patients (Figure 3C, $P = .003$). During the long-term follow-up, PTC patients with high expression levels of the Ki-67 labeling index had worse DFS compared with the low expression group (Figure 3D, $P = .006$). However, no significant difference was observed in Ki-67 mRNA expression level in tumor size ($P = .819$), tumor stage ($P = .357$), or overall survival (OS, $P = .476$), among thyroid cancer patients (Supplementary Figure S1).

In the GSE60542 dataset, a similar result was determined. Patients with regional lymph node metastasis had higher Ki-67 mRNA expression levels compared with lymph node-negative patients (Figure 4A). Besides, a higher Ki-67 mRNA expression level was also observed in patients with age >55 years at diagnosis and wild-type B-raf^{V600E} tumors (Figure 4B, 4C; $P = .041$, $P = .002$, respectively). Nonetheless, the primary tumor size ($P = .918$), stage ($P = .490$), multifocality ($P = .733$), and gender ($P = .707$) did not influence the expression levels of Ki-67 mRNA in thyroid cancer patients (Supplementary Figure S2). Figure 5 showed the immunohistochemical expression of Ki-67 in thyroid cancer tissues and normal tissues based on the Human Protein Atlas, which indicated that Ki-67 was stained in tumor cell nuclear.

Discussion

In this study, we validated some important risk factors mentioned in previous studies and take it a step further, as the Ki-67 labeling index was usually ignored in clinical studies for PTC. The CLNM was histopathologically confirmed in 197 (45.8%) PTC patients which was relatively higher than in some previous studies.[23,24] This divergence might be due to the different study populations and sample sizes. Additionally, an almost three-fold risk of CLNM was determined in PTC patients with male gender (OR = 3.020) or large tumor (>1 cm, OR = 2.808) and multifocality (OR = 2.079) displayed a nearly two-fold risk of CLNM. In our study, a high frequency of BRAF^{V600E} mutation was determined, which was similar to previous studies in the Chinese population,[25-27] but higher than that in most western patients.[28] This divergence could be contributed to the different genomic and transcriptomic characterization of the study population with varied ethnicities.[27] In our study, BRAF^{V600E} mutation which was reported as a risk factor in promoting CLNM[29] was not determined associated with CLNM in our study. This difference was potentially due to that approximately 16% of patients did not undergo the BRAF^{V600E} mutation test before the surgery which was a common limitation in recent research.[30,31] Interestingly, concerning the antithyroid antibodies, Wen et al[32] demonstrated that a single TgAb positive was a risk factor in inducing the CLNM in PTC patients with Hashimoto's thyroiditis while a single TPOAb positive was regarded as a protective factor in this subpopulation. Recently, 1 study with a larger population further confirmed the significant association between antithyroid antibody levels and CLNM in PTC patients.[31] In the present study, there was a significant difference in serum level of TgAb level among patients with CLNM or not (306.70 ± 739.80 vs 165.78 ± 365.96; $P = .044$), but it was not considered to be the risk factor in inducing the CLNM during the univariate analysis.

Regarding the molecular marker, the Ki-67 labeling index was a pivotal proliferative indicator in multiple solid malignancies, especially in terms of breast and lung cancer.[15,16] It could not only reflect the biological characteristics of the tumor but guide the postoperative adjuvant therapy. However, the utility of the Ki-67 labeling index in thyroid cancers was limited as a result of its generally low expression level in it. Nonetheless, reviewing recently published literature, some scholars have determined that a high expression level of Ki-67 (>10%) was correlated to lymph node (LN) metastasis and tumor recurrence in PTC.[20,33,34] Noticeably, with four

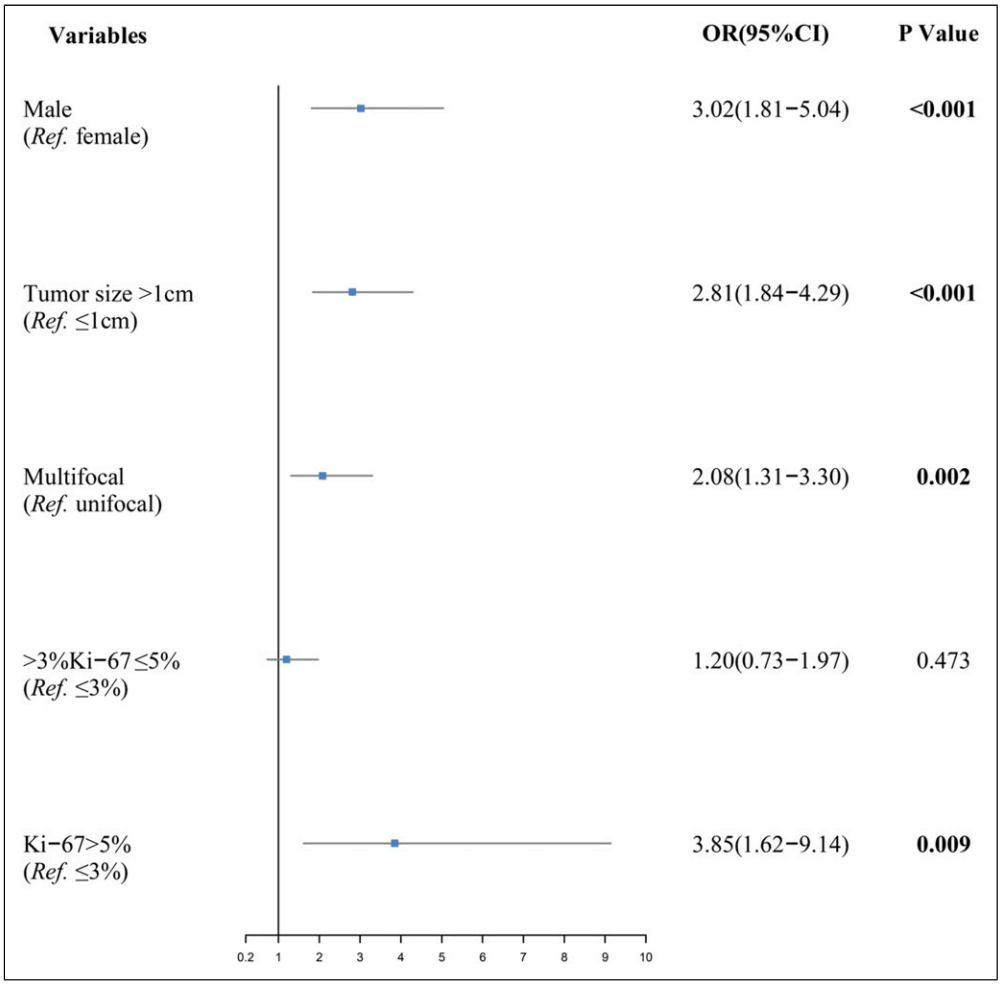

Figure 2. The forest plot of the multivariate logistic analysis for evaluating the independent clinical factors associated with CLNM. Ref: reference; OR: odd ratio; CI: confidence interval; CLNM: central lymph node metastasis.

Table 3. The Clinicopathological Characteristics of PTC Patients With CLNM Among Different Expression Levels of Ki-67.

		No. (%) of Patients					
Variable	Subgroup	≥0% and ≤3% (n = 111)	>3% and ≤5% (n = 43)	>5% (n = 18)	P_1	P_2	P_3
No. of CLNM	≥1 and <3	52 (47)	24 (56)	4 (22)	[a]0.293	[a]0.052	[a]0.050
	≥3 and <5	32 (29)	7 (16)	5 (28)			
	≥5	27 (24)	12 (28)	9 (50)			
Ratio of CLNM	>0 and ≤.25	33 (30)	19 (44)	1 (6)	[a]0.395	[b]**0.016**	[b]0.110
	>.25 and ≤.50	31 (28)	9 (21)	6 (33)			
	>.50 and ≤.75	18 (16)	5 (12)	3 (17)			
	>.75 and ≤1.00	29 (26)	10 (23)	8 (44)			

Note: Ratio of CLNM: positive central lymph nodes/harvested number of central lymph nodes, excluding patients with 1 or 2 harvested number of central lymph nodes.

P_1: ≥0% and ≤3% group compared with >3% and ≤5% group; P_2: ≥3% and ≤5% group compared with >5% group; P_3: ≥0% and ≤3% group compared with >5% group.

Bold values indicate statistical significance (P < .05).

Abbreviation: PTC: papillary thyroid carcinoma; CLNM: central lymph node metastasis.

[b]Two-tail Fisher exact test.

[a]Pearson's Chi-squared test.

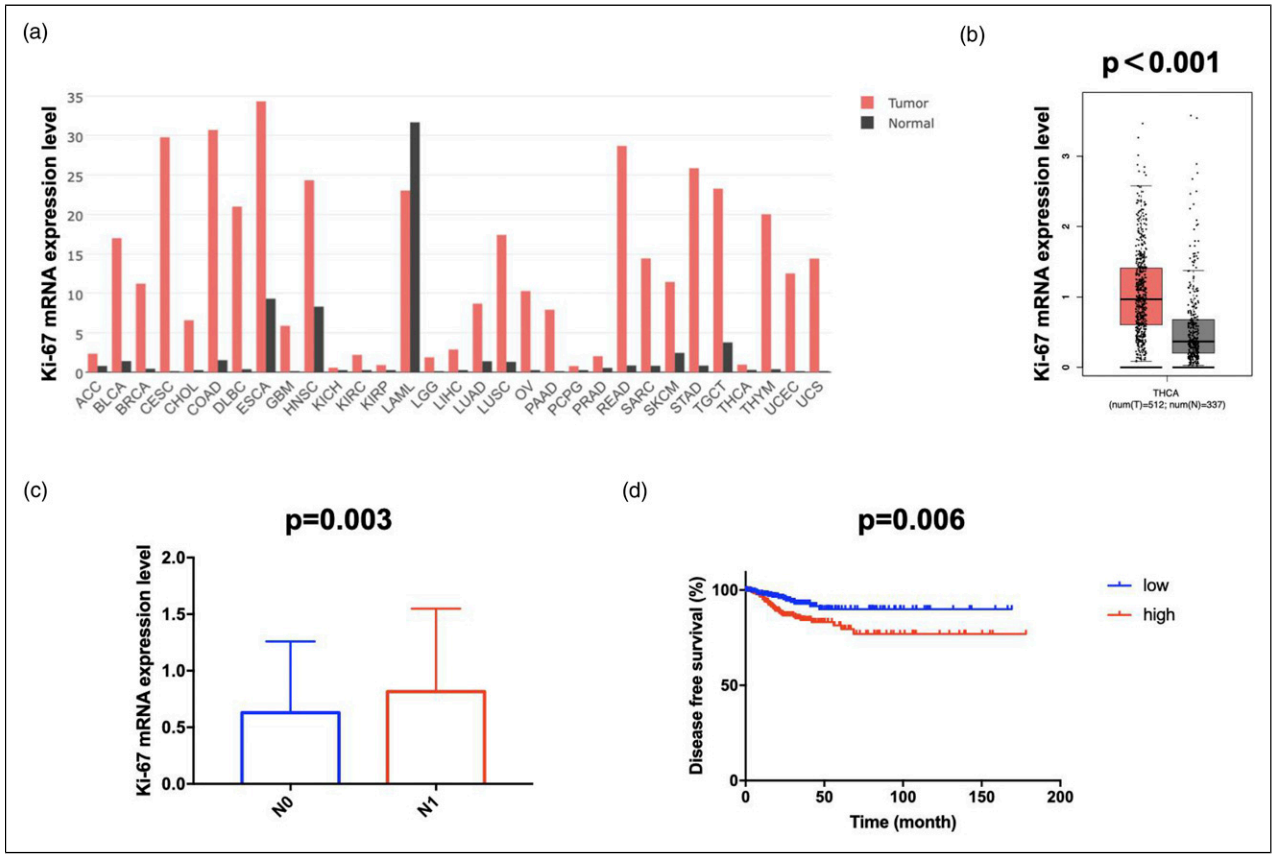

Figure 3. The characteristics of Ki-67 in the TCGA database. (a) The expression level profile of Ki-67 in multiple cancers. (b) The expression level of Ki-67 mRNA was higher in thyroid cancer tissues than in thyroid normal tissues. (c) Ki-67 expression in N0 and N1 group. (d) Association between DFS of thyroid cancer patients and Ki-67 mRNA expression from the TCGA database. ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangio carcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma.

hundred patient involvements, Matsuse and colleagues determined that the Ki-67 labeling index (>10%) combined with TERT promoter mutation could predict the DFS in PTC patients during the postoperative follow-up.[34] Furthermore, the findings of Lindfors et al. suggested that even 3% of Ki-67 expression was also significantly associated with the regional LNM and recurrence with a sensitivity of 50% and specificity of 80% ($P = .015$).[19] They also discovered a higher expression level of Ki-67 in patients with lymph node ratio (LNR) > 21% ($P = .023$) and extrathyroidal extension ($P = .001$) but not the N stage. In our study, patients with CLNM (+) were observed to have a higher Ki-67 labeling index than patients with CLNM (−). Moreover, a Ki-67 labeling index of >5% was significantly associated with the CLNM in PTC patients ($P = .009$). However, there was no general statistically significant association between the number of positive lymph nodes as well as LNR and the Ki-67 labeling index. We suggested the difference existed if the sample was expanded. For example, the volume of positive lymph nodes was a borderline negative correlation with the expression levels of Ki-67 (≥0% and ≤3% group vs Ki-67 > 5% group, $P = .050$).

Additionally, to determine the relationship between the Ki-67 labeling index and the prognostic of PTC patients, TCGA and GEO databases were further reviewed and analyzed. We observed that the level of Ki-67 is overexpressed in many kinds of cancers based on GEPIA. Although the Ki-67 expression level was much lower in thyroid cancer than in other cancers, which was significantly higher in thyroid cancer than in thyroid normal tissue in the TCGA database. Moreover, the expression level of the Ki-67 labeling index was associated with a higher N

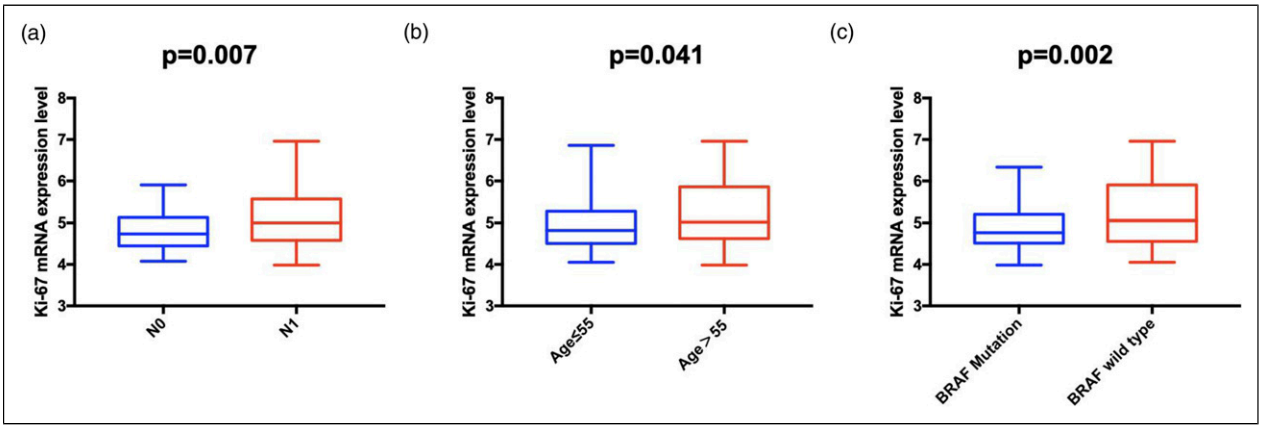

Figure 4. The clinicopathological significance of Ki-67 expression level in thyroid cancer in GSE60542. (a) The expression level of Ki-67 mRNA was higher in N1 than in the N0 group. (b) The expression level of Ki-67 mRNA was higher in the age>55 than in the age≤55 group (b). (c) The expression level of Ki-67 mRNA was higher in BRAF wild type than in the BRAF mutation group.

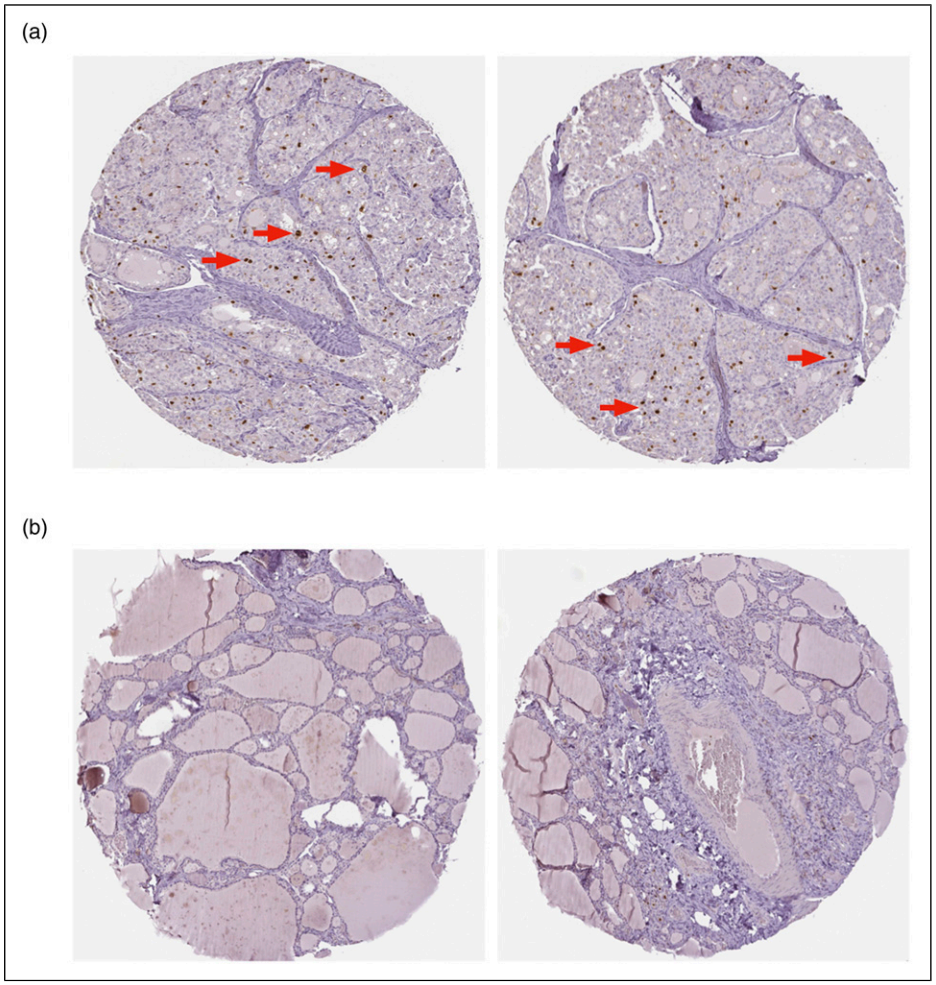

Figure 5. Immunohistochemical expression of Ki-67 in thyroid cancer tissues and normal tissues based on the Human Protein Atlas. (a) Thyroid cancer tissues. (b) Thyroid normal tissues.

stage, which was in accordance with our clinical findings. As for DFS, a worse DFS pattern was observed in thyroid cancer patients, which partially validated the conclusion from the preliminary study on this topic.[18] In 2010, Ito et al. investigated the role of the Ki-67 labeling index in the prognosis of PTC in Japan.[18] As they determined, the elevated expression level of the Ki-67 labeling index, even >1%, was an independent adverse prognostic factor for the DFS (HR = 4.13, 95% CI: 2.19-7.75).

Furthermore, we also found that the expression level of Ki-67 was higher in the BRAF wild-type group in the GSE60542 dataset. However, the relationship between Ki-67 and BRAF is poorly investigated, and further studies are warranted. Thus, the function and clinical value of Ki-67 were possibly neglected in thyroid cancer. We should pay more attention to the expression level of Ki-67 in clinical practice since it may play a role in helping to make better individualized clinical decisions, regardless of other clinicopathological features. By contrast, both studies made by Espenbetova et al[17] and Müssig et al[33] showed PTC patients with high Ki-67 labeling index had a more advanced stage. However, our results did not support this view. Consequently, whether the Ki-67 labeling index could influence the stage of PTC patients need further evaluation.

There is some strength needed to be mentioned. To our knowledge, this is the only one of the few studies in China to evaluate the association between the Ki-67 labeling index and the risk of CLNM in Chinses PTC patients. Besides, based on two large population-based genetical databases, the negative impact of the Ki-67 labeling index on the long-term DFS of PTC is further determined.

Nevertheless, we acknowledge that there are several limitations presented in our study. First, this is a retrospective single center-based study from China, which inevitably has resulted in a selection bias. Second, ultrasound characteristics and other basic information were missing in this study, which could be added in the following works. In addition, the distinguishment of ectopic thyroid tissue and metastatic thyroid carcinoma in central compartments needs further evaluation, as it might affect the actual CLNM rate in PTC.[35] Last, we only evaluated the role of the Ki-67 labeling index in the CLNM and the clinical outcome of PTC patients. Other biomarkers like TERT mutations were not evaluated in the present study, as only a few patients received these tests after hospitalization. For this reason, more molecular markers are expected to be evaluated to strengthen our results and better clarify the potential mechanisms of the biological behavior in PTC, especially in terms of the aggressive subtype.

Conclusion

In summary, a higher Ki-67 labeling index, especially >5%, is remarkably correlated with the CLNM in PTC patients. Besides, the Ki-67 labeling index is also observed to be associated with CLNM as well as DFS of PTC patients during the bioinformatic analyses. Thus, the Ki-67 labeling index may act as an important molecular marker of PTC patients and become prognostic indicators to enrich the well-established conventional TNM classification. Although Ki-67 is less well-studied, further studies are needed to further elucidate its clinical value in PTC.

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Author Contributions

(I) Conception and design: YM, DXH. (II) Administrative support: YM, DXH. (III) Provision of study materials or patients: YM. (IV) Collection and assembly of data: LY, XZ. (V) Data analysis and interpretation: LY, XZ. (VI) Manuscript writing: All authors. (VII) Final approval of manuscript: All authors.

Declaration of Conflicting Interests

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Ethics Approval and Consent to Participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for patients derived from our medical center was waived by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University in view of the retrospective nature of the study and all the procedures being performed were part of the routine care. As the molecular and genetic data were extracted from the TCGA and GEO databases, they did not contain any personally identifiable information. Informed consent and ethical approval were not required from the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID iD

Yu Min https://orcid.org/0000-0002-1266-3325

Supplemental Material

Supplemental material for this article is available online.

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