Effective Predictor Factors for Lymphy Node Metastasis and Survival in Patients with Betel Nut-Ralated Oral Squamous Cell Carcinoma

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Article

Effective Predictor Factors for Lymphy Node Metastasis and Survival in patients with Betel Nut-ralated Oral Squamous Cell Carcinoma

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Abstract: Background: Statistics from the Ministry of Health and Welfare reported oral cancer as one of the most prevalent malignant cancers, with the third highest incidence and the fifth leading cause of death among men in Taiwan. Lymph node metastasis in oral cancer usually has a low survival rate, with no significant improvement in the past 30 years. Therefore, a more effective survival predictor is warranted. Many cancer studies revealed that monitoring tumor thickness and lymph node density, in addition to tumor, node, and metastasis (TNM) stages, can provide more accurate predictions. **Methods:** This retrospective study analyzed data from 612 patients with oral cancer who had the habit of chewing betel nuts. The study focused on tumor thickness, lymph node density, and the regional distribution of lymph node metastasis to determine their effectiveness as predictors. **Results:** The results revealed that a tumor thickness of 6 mm indicated cervical lymph node metastasis and was the optimal cutoff point for overall survival. The optimal cutoff value for lymph node density was 0.04. Patients with a tumor thickness of >6 mm and a lymph node density of >0.04 had significantly lower overall survival rates. Additionally, patients with >1 lymph node metastasis level and lower cervical metastasis exhibited a relatively worse prognosis. **Conclusion:** Therefore, in addition to TNM staging, tumor thickness, lymph node density and metastasis level are suitable as a parameter for predictors that can be used as references for adjuvant therapies for better therapeutic effects.

Keywords: tumor thickness; lymph node density; lymph node metastasis level; oral cancer; survival rate

1. Introduction

Oral cancer is the most prevalent malignant head and neck cancer, characterized by cervical lymph node metastasis. The latest cancer registration reports from Taiwan revealed that oral cancer causes >3,000 deaths annually, a figure that has not decreased despite advances in medical treatments[1]. Regardless of the progression in surgical techniques and radiotherapy treatment diversity, the 5-year survival rate of oral cancer remains <50%, with particularly low survival rates among patients with lymph node metastasis[2,3]. The American Joint Committee on Cancer (AJCC) staging is most predominantly used for establishing treatment plans and predicting prognosis in Europe and the United States. However, the AJCC staging does not indicate the level of tumor invasion or predict lymph node metastasis. Thus, a more effective predictor is warranted in addition to AJCC staging. The 2008 version of the AJCC staging included tumor invasion depth into the T stage; however, the accurate tumor invasion depth is only available in postoperative pathological reports. Tumors with smaller sizes in the early stages may exhibit greater thickness and invasion depth, leading to their underestimation in tumor, node, and metastasis (TNM) staging[4]. Patients with no clinical lymph node metastasis exhibit an approximately 30% likelihood of having concealed lymph node metastasis. Additionally, greater tumor size indicates an increased chance of lymph node metastasis[5–7]. Tumor thickness not only represents the tumor size but also demonstrates how the

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tumor grows in a three-dimensional space, including both endophytic and exophytic growth. As tumor cells grow, the tumor's capillary proliferation tends to be closer to lymphatic vessels, increasing its likelihood of entering these vessels and the tumor's metastatic ability, thus making it an indicator of lymph node metastasis[8–10]. Han et al. revealed that pathological or radiological examinations of early tongue cancer rarely detect signs of lymph node metastasis. As a result, the patient's N stage is often underestimated, affecting the overall survival rate[11]. Therefore, a more effective lymph node metastasis predictor is warranted. So far, routine histopathologic examinations have not included lymph node density (number of metastatic lymph nodes/number of removed lymph nodes). Recent studies on bladder and esophageal cancers revealed that the use of lymph node density for survival rate prediction provides better results than traditional TNM staging[12,13], indicating lymph node density as an effective independent predictor. Studies on head and neck cancers revealed that lymph node density is a reliable predictor of survival rate; however, there are some analytical data and results are discrepancies and controversies[14,15]. An effective predictor should be objective, accessible, and readily comprehensible. To determine effective predictors for lymph node metastasis and overall survival rate, the present study investigated patients from a single medical center with the habit of betel nut chewing.

2. Materials and Methods

2.1. Patients

This study investigated tumor thickness, lymph node density, and lymph node metastasis area to determine their use as effective predictors. Participants included Betel Nut-chewing patients from the Department of Oral and Maxillofacial Surgery, Mackay Memorial Hospital, from December 2002 to December 2012. Inclusion criteria were patients with oral squamous cell carcinoma had a history of Betel Nut chewing which was defined as the behavioral use of Betel Nut for at least 10 y. Daily Betel Nut consumption about 10-25 quids and received surgical treatments, including extensive excision and cervical lymphatic dissection. The exclusion criteria were the unclear number of removed lymph nodes, distant metastasis, and having previously received radiotherapy or chemotherapy. Finally, this study included 612 patients and determined patients' staging based on the AJCC seventh edition. Pathological specialists interpreted the records to determine the number of metastatic lymph nodes, removed lymph nodes, determined the lymph node metastasis area, and calculated the lymph node density. Tumor thickness was measured as the vertical depth from the tumor surface to tumor cell invasion in connective tissue through the basement membrane.

2.2. Statistics

The area under the ROC curve (AUC) was 0.614, sensitivity was 70.91, and specificity was 60.50, indicating that a tumor thickness of 6 mm is the optimal cutoff value for all primary sites of oral cancer (Figure 1A). The AUC was 0.648, sensitivity was 69.32, specificity was 60.42, and lymph node density was 0.04 (Figure 1B). The Kaplan–Meier method was used to construct survival curves, and a univariate Cox regression model was established to estimate the impact of clinicopathological variables on survival. Only variables with a p-value <0.05 were considered statistically significant. Clinicopathological variables that were statistically significant in the univariate analysis were then included in a stepwise forward multivariate Cox regression analysis. Overall survival was defined as the time from the date of surgery to the date of death or the last follow-up. All tests were two-sided with a significance level set at $\alpha = 0.05$. A p-value of <0.05 was considered statistically significant for all analyses.

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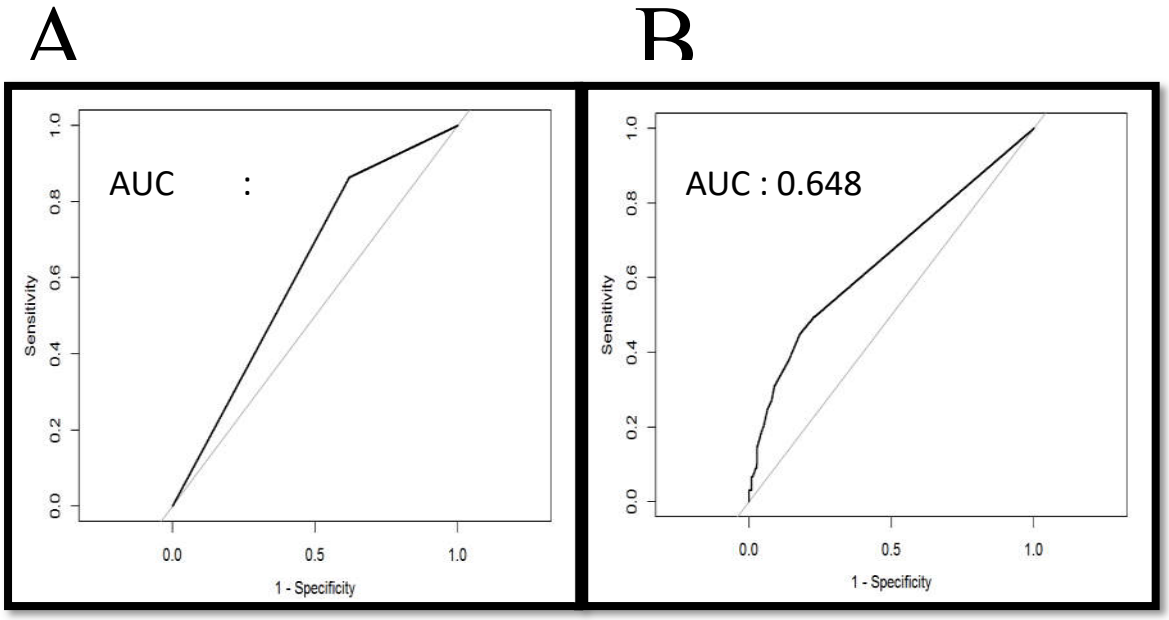

Figure 1. (A) The receiver operating characteristic curve for the tumor thickness value. The tumor thickness value of 6 mm is the optimal cutoff value. (B) The receiver operating characteristic curve for the lymph node density ratio. The lymph node density ratio of 0.04 is the optimal cutoff value.

3. Results

This study included 612 patients with oral cancer, including 568 males and 44 females, with an average age of 53.3 years. The buccal mucosa was the most prevalent tumor site, followed by the tongue. Table 1 shows other original data on clinical pathological parameters.

Table 1. Clinicopathological characteristics of the study participants.

Characteristics		No. of patients(%)
Gender	Female	44(7.2%)
	Male	568(92.8%)
Age	Mean	53.3 y/o
DM	No	486(79.4%)
	Yes	126(20.6%)
Tumor size	T1~T3	259(42.3%)
	T4	353(57.7%)
Lymph node stage	pN0	372(60.8%)
	pN+	240(39.2%)
Pathologic stage	I-III	583(95.3%)
	IV	29(4.7%)
Primary site	Buccal	246(40.2%)
	Tongue	170(27.7%)
	Other	206(32.1%)
Cell differentation	Well	219(35.8%)
	Moderate＋Poor	393(64.2%)
Lymphovascular invasion	No	500(81.7)
	Yes	112(18.3%)
Perineural invasion	No	479(78.3%)
	Yes	133(21.7%)
Extranodal extension	No	522(89.7%)
	Yes	60(10.3%)
Tumor thickness	≦6mm	133(30.5%)
	>6mm	305(69.5%)

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Lymph node density	≦0.04	514(84.0%)
	>0.04	98(16.0%)
Level of nodal metastasis	level I~III	223(92.9%)
	level IV~V	17(7.1%)
Survival status	Alive	414(67.7%)
	Dead	198(32.4%)
Follow-up	Mean	54.5 months

3.1. Cox Univariate and Multivariate Analyses of Overall Survival

In the univariate Cox analysis, several clinical factors were identified as significant predictors of overall survival in patients with OSCC(Table 2). These included higher lymph node density (HR 3.99, 95% CI 2.94–5.42, $p < 0.001$), the presence of lymph node metastasis (pN+) (HR 2.96, 95% CI 2.23–3.92, $p < 0.001$), larger tumor size (T4) (HR 3.31, 95% CI 2.31–4.63, $p < 0.001$), and perineural invasion (HR 2.70, 95% CI 2.00–3.67, $p < 0.001$). Additionally, diabetes mellitus (HR 2.59, 95% CI 1.94–3.47, $p < 0.001$), poor cell differentiation (HR 3.03, 95% CI 1.83–5.00, $p < 0.001$), lymphovascular invasion (HR 2.65, 95% CI 1.96–3.60, $p < 0.001$), and increased tumor thickness (>6 mm) (HR 3.89, 95% CI 2.24–6.71, $p < 0.001$) were associated with a significantly increased risk of poor survival. Covariates with a p-value <0.05 in the univariate analysis were further evaluated in the multivariate Cox analysis to assess their independent prognostic significance. Covariates with a p-value <0.05 in the univariate analysis were further assessed using multivariate Cox analysis to determine their independent prognostic value. The multivariate analysis confirmed that higher lymph node density (HR 2.18, 95% CI 1.11–4.28, $p = 0.024$), lymph node metastasis (HR 1.83, 95% CI 1.01–3.34, $p = 0.048$), larger tumor size (HR 2.29, 95% CI 2.16–4.35, $p < 0.001$), the presence of diabetes (HR 2.96, 95% CI 1.71–5.13, $p < 0.001$), perineural invasion (HR 2.32, 95% CI 1.60–3.36, $p < 0.001$), and increased tumor thickness (HR 2.95, 95% CI 1.59–5.47, $p = 0.001$) were independent risk factors associated with decreased overall survival. These findings underscore the importance of these variables in predicting patient prognosis and highlight their potential role in guiding clinical decision-making and treatment strategies for OSCC patients.

Table 2. Univariate and multivariate analyses of overall survival in patients with OSCC.

Variable	**Univariate analysis**		Multivariate analysis	
	HR(95%CI)	p-value	HR(95%CI)	p-value
Lymph node density				
≦0.04 vs >0.04	3.99(2.94-5.42)	<0.001	2.18(1.11-4.28)	0.024
Lymph node status				
pN0 vs pN+	2.96(2.23-3.92)	<0.001	1.83(1.01-3.34)	0.048
Tumor size				
T1-3 vs T4	3.31(2.31-4.63)	<0.001	2.29(2.16-4.35)	<0.001
Diabetes mellitus				
No vs Yes	2.59(1.94-3.47)	<0.001	2.96(1.71-5.13)	<0.001
Gender				
Female vs Male	1.46(0.89-2.40)	0.138		
Cell differentiation				
Well vs Moderate +Poor	3.03(1.83-5.00)	<0.001		
Perineural invasion				
No vs Yes	2.70(2.00-3.67)	<0.001	2.32(1.60-3.36)	<0.001
Lymphovascular invasion				
No vs Yes	2.65(1.96-3.60)	<0.001		
Tumor thickness				
≦6mm vs >6mm	3.89(2.24-6.71)	<0.001	2.95(1.59-5.47)	0.001

3.2. Association Among Tumor Thickness, Lymph Node Metastasis, and Overall Survival Rate

A total of 213 (48.6%) and 225 (51.4%) patients were positive and negative for lymph node metastasis, respectively. Figure 2A shows the correlation distribution between tumor thickness and lymph node metastasis. Tumor thickness was ≦6 mm for 44 (20.7%) and >6 mm for 169 (79.3%) of

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the 213 patients with lymph node metastasis, with a significant difference between them ($p < 0.001$, Table 3). Our results revealed that a tumor thickness of 6 mm is crucial for predicting regional lymph node metastasis. Different tumor site were further analyzed. A total of 60 (49.6%) and 61 (50.4%) patients were positive and negative for lymph node metastasis in the tongue (9 mm) ($p < 0.001$, Table 3). respectively. Figure 2B shows the correlation distribution between lymph node metastasis and tumor thickness.. A total of 89 (50.6%) and 87 (49.4%) patients were positive and negative for lymph node metastasis in the buccal mucosa (7 mm) ($p < 0.001$, Table 3), respectively. Figure 2C shows the correlation distribution between lymph node metastasis and tumor thickness.. The Kaplan–Meier curve analysis, comparing patients with a tumor thickness of ≦6 mm to those with a tumor thickness of >6 mm, revealed poorer overall survival among patients with a tumor thickness of >6 mm ($p < 0.001$, Figure 3A). After including tumor thickness in the analysis, patients with lymph node metastasis and a tumor thickness of >6 mm demonstrated poorer overall survival ($p = 0.006$, Figure 3B), indicating tumor thickness as an effective predictor of survival rate.

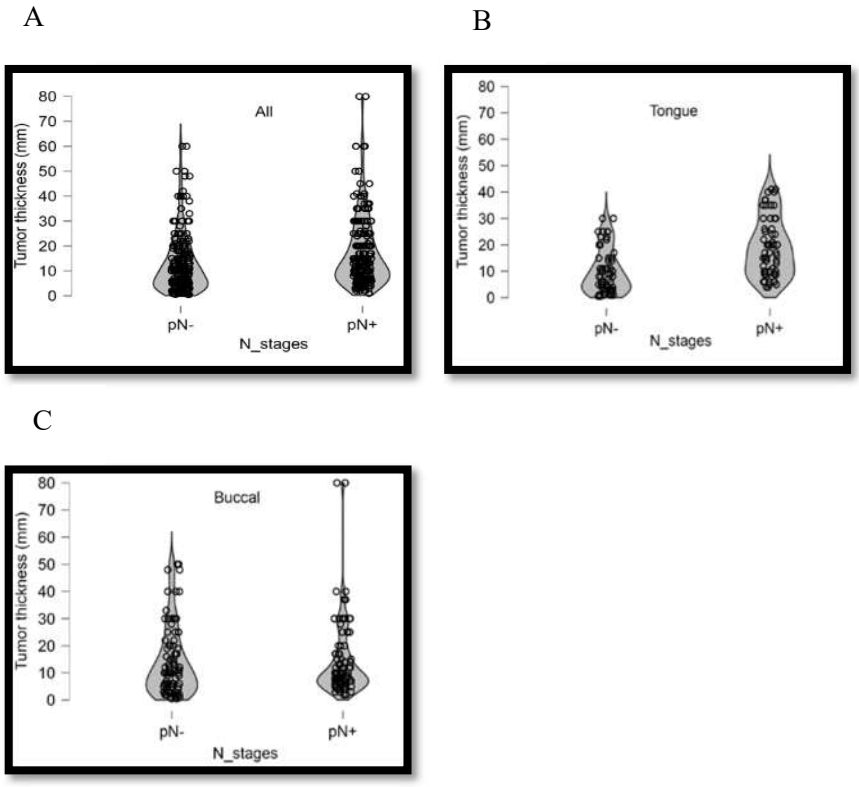

Figure 2. (A) Correlation distributions between tumor thickness and lymph node metastasis for cancers of all anatomic sites. (B, C) Correlation distributions between tumor thickness and lymph node metastasis for cancers of different anatomic sites.

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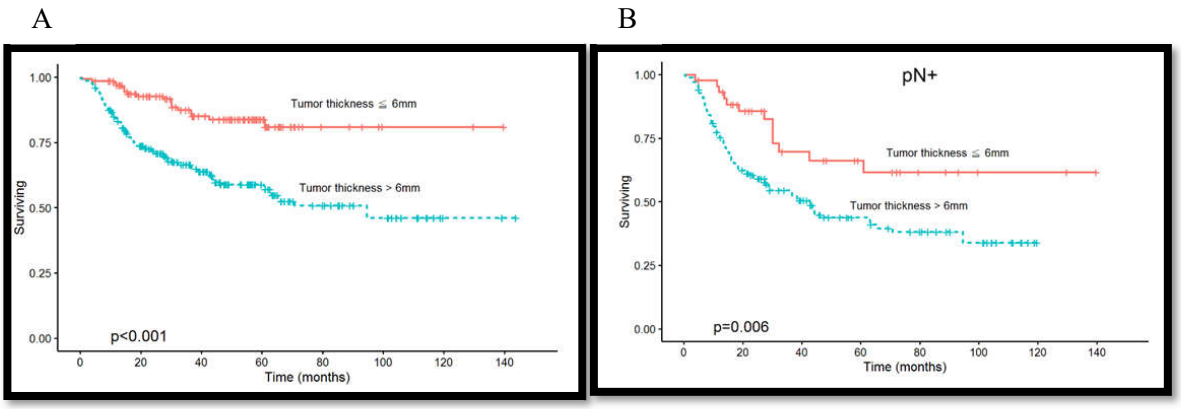

Figure 3. (A) Kaplan–Meier survival rate curve; comparison between patients with a tumor thickness of >6 mm and ≦6 mm, where patients with a tumor thickness of >6 mm demonstrated poorer overall survival (p < 0.001). (B) Patients with lymph node metastasis and tumor thickness of >6 mm demonstrated poorer overall survival rates after including tumor thickness in survival rate analysis (p = 0.006).

Table 3. Association between tumor thickness at different tumor sites and lymph node metastasis.

Primary site		pN0(%)	pN+(%)	p-value
All	Tumor thickness≦6mm	89(39.6%)	44(20.7%)	<0.001
	Tumor thickness >6mm	136(60.4%)	169(79.3%)	
Tongue	Tumor thickness≦9mm	33(54.1%)	12(20.0%)	<0.001
	Tumor thickness >9mm	28(45.9%)	48(80.0%)	
Buccal	Tumor thickness≦7mm	35(40.2%)	23(25.8%)	<0.001
	Tumor thickness >7mm	52(59.8%)	66(74.2%)	

3.3. Association between Clinical Pathological Parameters and Tumor Thickness

Univariate and multivariate analyses of clinicopathological parameters effect factors for tumor thickness. Result revealed that perineural invasion (p < 0.003), tumor size (p < 0.001), pathological stage (p < 0.001), and cell differentiation (especially moderate and poor differentiation) (p < 0.004) are independent factors affecting tumor thickness (Table 4).

Table 4. Univariate and multivariate analyses of clinicopathological parameters effect factors for tumor thickness.

Variables	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Lymphovascular invasion				
No	Reference			
Yes	2.97(1.64-5.39)	<0.001		
Perineural invasion				
No	Reference		Reference	
Yes	3.99 (2.18-7.31)	<0.001	2.75 (1.41-5.36)	<0.003
Tumor size				
T1-T3	Reference		Reference	
T4	8.25 (5.12-13.28)	<0.001	2.55 (1.13-5.74)	<0.001
Pathologic stage				
I-III	Reference		Reference	
IV	1.75 (1.37-2.22)	<0.001	4.13 (1.87-9.11)	<0.001
Level of nodal metastasis				
Level I-III	Reference			
Level IV-V	1.06(0.26-4.23)	0.929		
Involved level				

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1 Level	Reference			
2 Levels	1.39(0.59-3.25)	0.442		
>2 Levels	2.22(0.47-10.31)	0.308		
Extranodal extension				
No	Reference			
Yes	3.68(1.62-8.35)	0.002		
Diabetes mellitus				
No	Reference			
Yes	2.40(1.32-4.38)	0.004		
Cell differentation				
Well	Reference		Reference	
Moderate＋Poor	1.94 (1.27-2.97)	<0.002	2.08 (1.25-3.467)	<0.004

3.4. Association between Lymph Node Density and Overall Survival Rate

Lymph node densities of ≦0.04 and >0.04 were observed in 514 (84.0%) and 98 (16.0%) patients, respectively. Additionally, 143 (59.4%) and 97 (40.6%) patients demonstrated lymph node metastases and lymph node densities of ≦0.04 and >0.04, respectively. Figure 4A shows the correlation distribution between lymph node metastasis and lymph node density. Figure 4B shows the correlation distribution between lymph node metastasis stage and density. The Kaplan–Meier curve analysis, when setting lymph node density 0.04 as the optimal cutoff value, revealed that patients with a lymph node density of >0.04 demonstrated poorer overall survival compared to those with a lymph node density of ≦0.04 ($p < 0.001$, Figure 5A). Patients with lymph node metastasis and density of >0.04 showed poorer overall survival rates when the lymph node density was included for the analysis of lymph node metastasis ($p < 0.001$, Figure 5B). Lymph node density remained a predictor for overall survival rate after including lymph node density in the analysis of the lymph node metastasis stage ($p < 0.001$, Figure 5C).

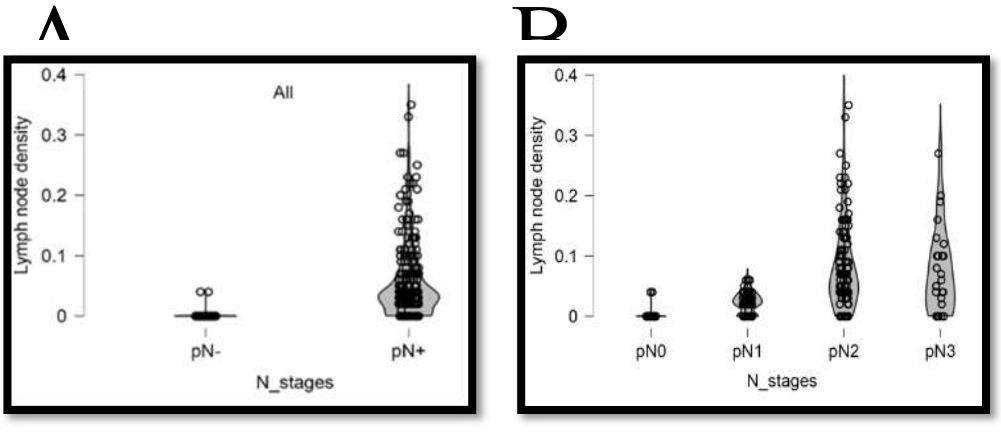

Figure 4. (A,B) Correlation distribution diagram between patients with lymph node metastasis and lymph node density. Lymph node metastasis cases are divided into pN1, pN2, and pN3 stages. Correlation distribution diagram between lymph node metastasis stage and lymph node density.

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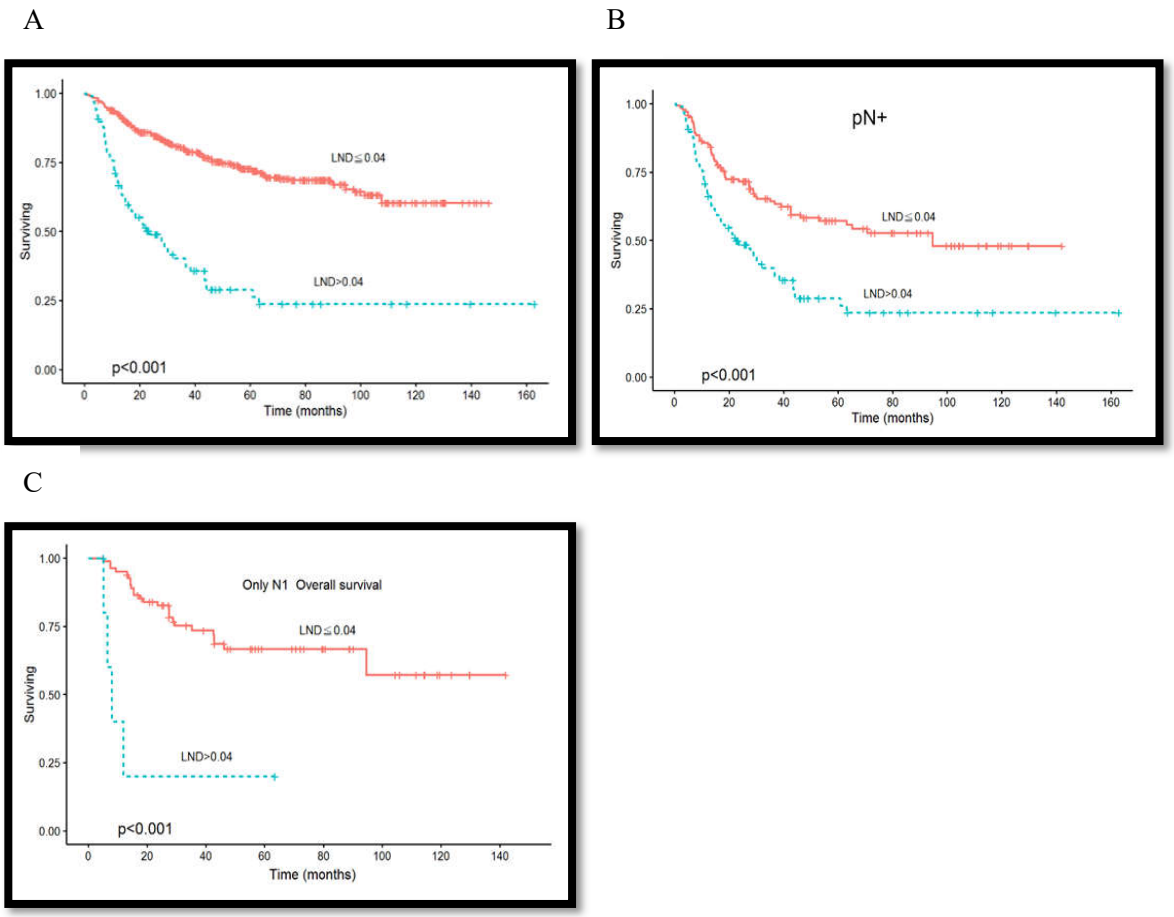

Figure 5. (A) Kaplan–Meier survival rate curve; comparison between patients with lymph node density of >0.04 and ≦0.04, where patients with a lymph node density of >0.04 exhibited poorer overall survival ($p < 0.001$). (B) Patients with lymph node metastasis and lymph node density of >0.04 demonstrated a poorer overall survival rate after including lymph node density in survival rate analysis ($p < 0.001$). (C) Patients in the pN1 stage with a lymph node density of >0.04 exhibited poorer overall survival rates by dividing the patients with lymph node metastasis into the pN1, pN2, and pN3 groups and including lymph node density into survival rate analysis of patients of different lymph node metastasis stages ($p < 0.001$). No significant difference was found between patients with pN2 and those with pN3.

3.5. Association between Clinical Pathological Parameters and Lymph Node Density

Univariate and multivariate analyses of clinicopathological parameters effect factors for lymph node density. Result revealed lymphovascular invasion ($p < 0.001$), tumor thickness ($p < 0.044$), Diabetes mellitus ($p < 0.011$) and cell differentiation , including moderate and poor differentiation ($p < 0.027$) (Table 5), as independent factors affecting lymph node density.

Table 5. Univariate and multivariate analyses of clinicopathological parameters effect factors for lymph node density.

Variables	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Lymphovascular invasion				
N0	Reference		Reference	
Yes	6.84(4.24-11.01)	<0.001	4.84(2.58-8.22)	<0.001
Perineural invasion				
No	Reference			
Yes	3.29(2.08-5.22)	<0.001		

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Tumor size				
T1-T3	Reference			
T4	1.61(1.02-2.548)	0.041		
Pathologic stage				
I-III	Reference			
IV	1.57(0.95-2.58)	0.073		
Level of nodal metastasis				
Level I-III	Reference			
Level IV-V	2.28(0.79-6.60)	0.126		
Involved level				
1 Level	Reference			
2 Levels	5.98(3.10-11.53)	<0.001		
>2 Levels	9.28(3.34-25.72)	<0.001		
Extranodal extension				
No	Reference			
Yes	17.87(9.75-32.76)	<0.001		
Tumor thickness				
≦6mm	Reference		Reference	
>6mm	2.92(1.56-5.49)	<0.001	1.98(1.02-3.86)	<0.044
Diabetes mellitus				
No	Reference		Reference	
Yes	2.16(1.34-3.49)	<0.002	2.09(1.18-3.86)	<0.011
Differentation				
Well	Reference		Reference	
Moderate＋Poor	2.46(1.46-4.15)	<0.001	1.987(1.08-3.64)	<0.027

3.6. Association between Lymph Node Metastasis Level and Overall Survival Rate

In the univariate and multivariate Cox analysis of associated mortality according to different levels metastasis of cervical lymph nodes in pN+ patients with OSCC. Level of nodal metastasis was identified as significant predictors of overall survival in pN+ patients with OSCC (Table 6). The Kaplan–Meier curve analysis revealed a poor overall survival rate regardless of lymph node metastasis level ($p < 0.001$, Figure 6A). Further analysis where patients were grouped based on lymph node metastasis level , all patients revealed whose lymph node metastasis was in the lower neck level ($p < 0.001$, Figure 6B) demonstrated a poorer survival rate.

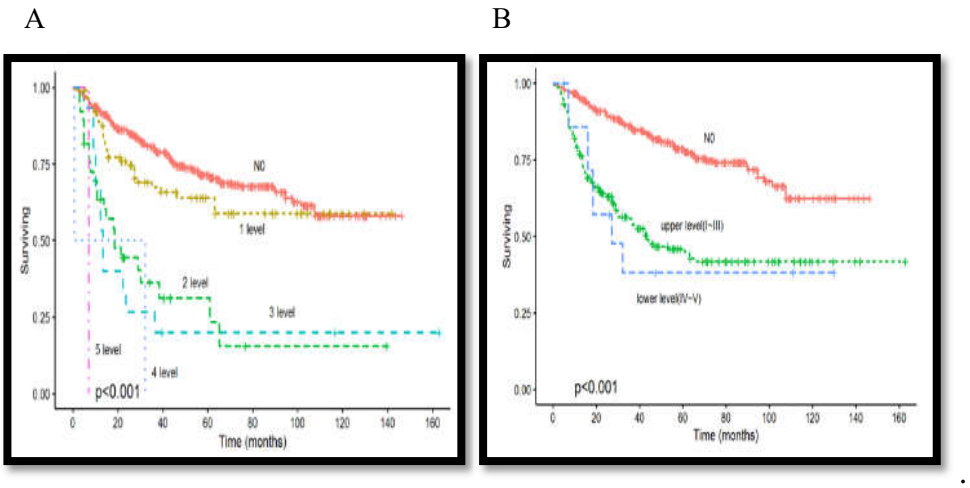

Figure 6. (A) Kaplan–Meier lymph node metastasis level distribution curve and overall survival rate. The results revealed poor overall survival rates, regardless of lymph node metastasis level ($p < 0.001$). (B) Further analysis revealed that patients with lymph node metastasis in lower cervical level (level VI-V) ($p < 0.001$) demonstrated poorer survival rates.

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Table 6. Analyses of associated mortality according to different levels metastasis of cervical lymph nodes in pN+ patients.

Variables	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Level of nodal metastasis				
I-III	Reference		Reference	
IV-V	1.87(1.06-3.30)	0.03	0.11(0.03-8.22)	0.003
Involved level				
1 Level	Reference			
2 Levels	2.22(1.46-3.38)	<0.001		
>2 Levels	2.23(1.27-3.83)	0.01		

3.7. Association between Overall Survival Rate and Tumor Thickness Combined with Lymph Node Density

Patients with a lymph node density of >0.04 and a tumor thickness of >6 mm demonstrated poorer overall survival when including lymph node density and tumor thickness simultaneously in survival rate analysis (p = 0.005, Figure 7A). Linear regression analysis revealed a positive correlation between tumor thickness and lymph node density (p = 0.0053) (Figure 7B).

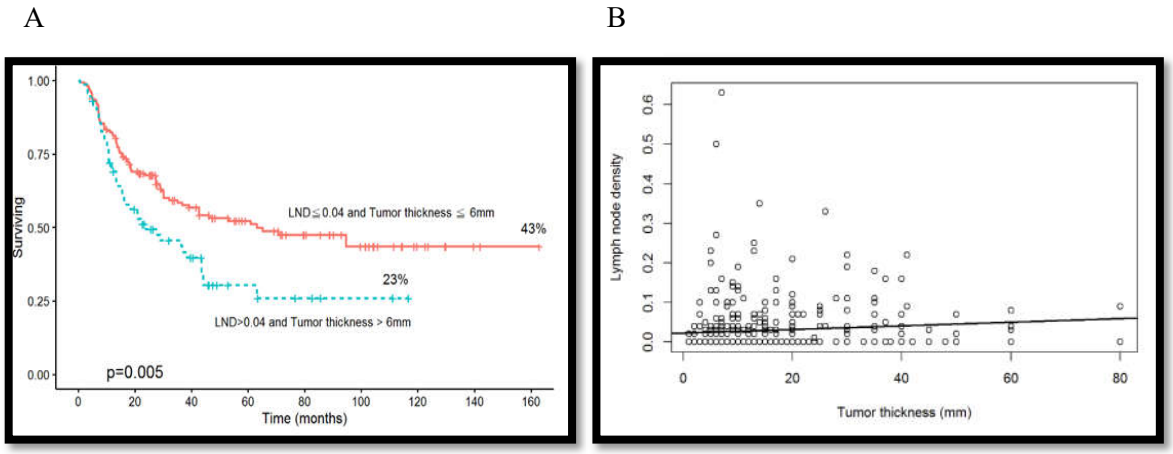

Figure 7. (A) After including both lymph node density and tumor thickness for survival rate analysis, patients with a lymph node density of >0.04 and tumor thickness of >6 mm demonstrated poorer overall survival rates (p = 0.005). Linear regression was utilized to examine the correlation between the overall survival rate and the two parameters of tumor thickness and lymph node density (p = 0.0053).

4. Discussion

TNM staging is a widely used method for cancer prognosis prediction[16]. However, this method often causes errors in predicting the cancer course in some patients, making the reliability of using the TNM staging for prognosis prediction questionable[17]. Previous studies identified tumor thickness as a predictor for lymph node metastasis[7,10,18], and the values of tumor thickness ranged from 2 to 10 mm in different studies[19,20].The significant variation in tumor thickness may be due to unclear tumor thickness definitions and a lack of a standardized measuring method[21]. Many researchers have attempted to determine the thickness value for metastatic capacity, but a consensus has not been reached yet. Giacomara proposed the invasion depth as the tumor range beneath the basement membrane[22]. However, the tumor thickness includes both invasion depth and tumor above the basement membrane and is the measured value of the entire tumor[22]. Breslow was the first researcher to study tumor thickness. He analyzed cutaneous melanoma in 1975 and revealed that different tumor thicknesses affected lymph node metastasis[23]. In 1986, Spiro and Mohit-

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Tabatabai et al. studied the correlation between oral cancer tumor thickness and lymph node metastasis for the first time[18,24]. Asakage in 1998, Kurokawa in 2002, O-Charoenrat in 2003, and Mücke in 2016 then conducted similar studies, confirming tumor thickness as a predictor for lymph node metastasis[19,20,25,26]. Mohit-Tabatabai et al. conducted a retrospective study that included 84 patients with early-stage oral floor carcinoma and indicated that preventive neck dissection was recommended for cN0 patients with a tumor thickness of >1.5 mm[24]. The retrospective study conducted by Asakage included 44 patients with early-stage tongue cancer. The study revealed 4 mm as the optimal cutoff value, showing statistical significance for lymph node metastasis and disease-free survival rate. Consequently, neck dissection was recommended for early-stage patients with a tumor thickness of >4 mm[25]. Mücke et al. revealed 8 mm as the optimal cutoff value for tongue cancer and recommended using tumor thickness to predict concealed lymph node metastasis[26]. However, Brown and Morton revealed no correlation between tumor thickness and lymph node metastasis[27,28]. The present study revealed that a tumor thickness of 6 mm is the crucial cutoff value for lymph node metastasis, with statistical significance when analyzing the overall survival rate. Moreover, the cutoff values for buccal mucosa and tongue were 7 and 9 mm for different tumor sites, respectively. Our data revealed certain differences from data obtained from overseas studies, which could be attributed to varying cancer risk factors in different countries, tumorigenic sites, and cell differentiation[29]. Patients included in this study were mainly T3–T4 and had the habit of betel nut chewing, with common tumorigenic sites of buccal mucosa and tongue. Betel nut chewing affects tumor thickness as repeated chewing wears out the oral mucosa, and the chronic irritation easily causes exophytic and ulcerative tumor patterns, resulting in tumor proliferation and thickening[30]. Wong et al. applied betel nut extract to hamster cheek pouches and observed increased tumor thickness, indicating that betel nut extract induces cancer and promotes tumor growth[31]. Liao confirmed that cancer cells are mainly presented poorly differentiated cells in countries where betel nut chewing is popular[32]. Huang et al. revealed that moderately and poorly differentiated cancer cells manifest lymph node metastasis[33].

The results of the present study revealed that cell differentiation level, especially moderate and poor differentiation, affects tumor thickness. Thus, our research results can be applied clinically in the following ways. 1. Preoperative imaging including MRI,CT and Sonography required measurements of tumor thickness are recommended to determine whether cervical lymph dissection is required. 2. surgeons can use this as a tool to identify the surgical range of regional lymph nodes for early-stage cancer and clinical N0 patients. For patients with tumor thickness greater than 6 mm, selective neck dissection is strongly recommended.

The key factor affecting cancer prognosis is cervical lymph nodes; however, the traditional N staging is not fully capable of prediction. Integrating lymph node density into the traditional TNM staging system could result in more accurate staging and better prognostic assessments, particularly for N+ patients. For example, in oropharyngeal cancer patients with pN1 and pN2 staging, if the number of metastatic lymph nodes exceeds four, but less than four are removed due to inadequate neck dissection, the staging may underestimate the pN1. Therefore, integrating lymph node density into the traditional TNM staging system, especially for lymph node staging, can more effectively predict patient prognosis. Studies on bladder, colorectal, breast, and cervical cancers have identified lymph node density as an effective prognostic predictor[14,34-36]. Kim et al. investigated the records of 211 patients with oral cancer to analyze the correlation between lymph node density and survival rate and revealed that patients with a lymph node density of >0.06 demonstrated poorer survival rates[37]. A random-effect model was used for statistical analysis in an international research alliance that included patients with oral cancer from 11 medical centers. This analysis, accounting for data heterogeneity and varying results from individual hospitals, revealed that a lymph node density of >0.06 is usually associated with a poor survival rate of patients, confirming lymph node density as an independent predictor of survival rate[38]. Some factors may affect the ratio of lymph node density, such as the neck dissection procedure, the interpretive ability of pathologists, and the quality of histopathologic slide. Inadequate neck dissection will reduce the total number of lymph nodes removed, increase the chance of occult metastasis, and lead to underestimation of the pathological

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stage. This results in higher lymph node density ratio, ultimately reducing the survival rate of patients[39]. The lymph node density is 0.04 by assuming one patient has one metastatic lymph node (24 total removed lymph nodes), and the lymph node density is 0.2 for another patient with one metastatic lymph node (but 5 total removed lymph nodes). Both patients have the N1 lymph node stage, but those with only five removed lymph nodes need to worry about the risk of occult metastasis and possibly underestimate the N staging.

The advantage of using lymph node density include : 1. Consideration of tumor factors : The number of metastatic lymph nodes. 2. Surgical treatment factors: The total number of lymph nodes removed during neck dissection. 3. Tumor staging factors : Pathologists need to carefully identify all lymph nodes and metastases. Another benefit of lymph node density is its usefulness as a quality indicator when performing neck dissection. The total removed lymph node number can be increased to reduce the chance of occult metastasis when including lymph node density as a surgical quality indicator, thereby improving the patient's survival rate.

This study revealed a lymph node density of 0.04 as the optimal cutoff value. Patients with pN+ and a lymph node density greater than 0.04 demonstrated a poorer prognosis. Therefore, based on previous studies and our findings, Integrating lymph node density into the traditional N staging in more accurately predicting patient prognosis. Moreover, the overall survival rate was poor when more than one metastasis level was observed or when the metastasis was in the lower neck level.

5. Conclusions

The advantages of this study are that all participants were from the same medical center and that >600 oral cancer patients with the habit of betel nut chewing underwent the same type of neck dissections based on therapeutic standards, thereby adequately representing sufficient local samples in Taiwan. Our results indicated that lymph node density, tumor thickness, and regional distribution of lymph node metastases are effective prognostic predictors that can be used as references for adjuvant therapies for the better overall survival of patients. Future studies warrant the use of a larger sample size to determine more effective predictors. For example, the correlation between tumor-infiltrating lymphocytes and the depth of tumor invasion can be analyzed .

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