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*Original Article*

# Relation of Depth of Invasion with Lymph Node Metastasis in Advanced Oral Cavity Squamous Cell Carcinoma

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**Abstract:**

***Background:*** *Depth of invasion (DOI) is a key histopathological marker in oral cavity squamous cell carcinoma (OCSCC), indicating tumor aggressiveness and predicting cervical lymph node metastasis, particularly in advanced cases with higher occult spread risk. As early-stage (T1, T2) tumors have well-documented patterns and predictable outcomes, this study focuses on advanced-stage (T3, T4) OCSCC, where DOI shows greater variability and stronger influence on staging and treatment planning.*

***Methods:*** *This cross-sectional study was conducted in the Department of Otolaryngology-Head & Neck Surgery, Bangladesh Medical University, Dhaka and National Institute of Ear, Nose and Throat, Dhaka for 18 months. A total number of 30 cases of advanced oral cavity Squamous cell carcinoma were enrolled for the study on the basis of inclusion and exclusion criteria. Post operative histopathological Depth of invasion were analyzed to predict the cervical node metastasis. Results of the study were expressed as mean, standard deviation (± SD), frequency and percentages. Unpaired students t-test and chi-square test was performed as applicable. p-value < 0.05 was accepted as level of significance.*

***Results:*** *Most patients (mean age 45 years, Male: Female = 2.75:1) were from rural areas, with tongue and buccal mucosa commonly affected. Lymph node metastasis occurred in 56.7% of patients, with a significant association between poor differentiation and nodal spread (P = 0.013). Depth of invasion (DOI) ranged from 1–13 mm (mean*

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*7.23 ± 1.87 mm). Lymph node metastasis was highest (91.7%) in tumors with DOI >10 mm (p = 0.002). Mean DOI was significantly greater in LN-positive patients compared to LN-negative cases (p = 0.000).*

***Conclusion:** Depth of invasion is strongly related with cervical lymph node metastasis and serves as a reliable predictor for staging, surgical planning, and prognosis in OCSCC. Incorporating DOI into routine histopathology and imaging can improve treatment decisions and patient outcomes.*

***Key words:** Oral cavity squamous cell carcinoma (T3, T4), Depth of invasion, Lymph node metastasis.*

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## Introduction:

Oral cancer is a major global health concern, frequently diagnosed at advanced stages despite a well-understood progression[1]. The rising incidence is largely attributed to changing lifestyle patterns, including tobacco and alcohol consumption. Oral cavity squamous cell carcinoma (OCSCC) primarily affects men in their 60s and 70s, often involving the lateral tongue and floor of the mouth[2]. Advanced-stage OCSCC (Stage III–IV) is characterized by aggressive local invasion and a high tendency to spread to regional lymph nodes, which significantly complicates management and reduces survival by approximately fifty percent[3].

Depth of invasion (DOI) has emerged as a reliable histological marker for cervical nodal metastasis[4]. The 8th edition of the AJCC staging manual now incorporates DOI as a key determinant for T-staging, reflecting its clinical significance[5]. While tumor size is a known predictor of outcomes, DOI provides a more precise assessment of tumor aggressiveness than total tumor thickness[6]. Advanced cases (T3 and T4) present more significant challenges as they often exhibit occult nodal spread[7,8]. Although early-stage (T1–T2) outcomes are more predictable, the diversity of T3 tumors makes DOI a critical independent predictor of survival in these populations[9].

Despite its importance, limited regional data exists regarding the specific relationship between DOI and nodal metastasis in advanced-stage cases in South Asia. Identifying clear DOI thresholds is essential for guiding clinical decisions, particularly concerning elective neck dissection [7]. This study aims to evaluate the correlation between post-operative histopathological DOI and cervical lymph node involvement in patients with advanced OCSCC to improve regional staging and prognostic accuracy.

## Methods:

This study was conducted using a cross-sectional design at the Department of Otolaryngology-Head & Neck Surgery of Bangladesh Medical University and the National Institute of Ear, Nose and Throat in Dhaka. The study period spanned 18 months, from January 2024 to June 2025. Ethical clearance was obtained from the Institutional Review Board (IRB) of the university before commencement. A total of 30 patients diagnosed with advanced (T3, T4) oral cavity squamous cell carcinoma were selected using purposive sampling based on specific inclusion and exclusion criteria. Written informed consent was obtained from all participants after explaining the study objectives and procedures in their native language.

Primary tumor samples and neck dissection specimens were collected post-operatively for histopathological analysis. Depth of invasion was measured perpendicularly from the reconstructed basement membrane of the adjacent normal mucosa to the deepest point of tumor invasion using the "plumb line" method as defined by AJCC guidelines. Tumors were also graded as well, moderately, or poorly differentiated according to the WHO classification. Lymph node status was assessed for levels I through V. Data were compiled and analyzed using SPSS version 26 and Microsoft Excel. Continuous data were expressed as mean and standard deviation, while categorical data were presented as frequencies and percentages. Statistical significance was determined using unpaired Student's t-tests and Chi-square tests, with a p-value of less than 0.05 considered significant.

**Results:**

The mean age of the study population was 45.08 ± 11.97 years, with a significant male predominance (M:F ratio 2.75:1; p = 0.011). Most patients (63.3%) resided in rural areas. Common risk factors included betel quid chewing (60.0%) and smoking (53.3%). The tongue was the most frequent primary tumor site (47%), followed by the buccal mucosa (37%) (Table -I).

Lymph node metastasis was present in 17 patients (56.7%), primarily affecting Level II nodes (41.1%). A significant association was found between tumor differentiation and nodal involvement; 88% of poorly differentiated tumors were node-positive compared to well-differentiated cases (p = 0.0128) (Table-II).

**Table I**

*Baseline Clinical and Demographic Profile and Risk Factors of the Study Population (n=30)*

| Characteristics | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| **Age (Years)** | < 40 | 12 | 40.0% |
| | > 40 | 18 | 60.0% |
| | Mean ± SD | 45.08 ± 11.97 | - |
| **Gender** | Male | 22 | 73.3% |
| | Female | 8 | 26.7% |
| **Residence** | Rural | 19 | 63.3% |
| | Urban | 11 | 36.7% |
| **Risk Factors*** | Betel Quid Chewing | 18 | 60.0% |
| | Smoking | 16 | 53.3% |
| | Poor Oral Hygiene | 15 | 50.0% |
| | Spicy Food Intake | 10 | 33.3% |
| | Ill-fitting Dentures | 9 | 30.0% |
| **Primary Site** | Tongue | 14 | 46.7% |
| | Buccal Mucosa | 11 | 36.7% |
| | Retromolar Trigone | 2 | 6.7% |
| | Others (Gingiva/Floor) | 3 | 10.0% |

* Multiple response

**Table II**

*Clinicopathological Characteristics of Lymph Node Metastasis (n=17)*

| Characteristics | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| **Differentiation** | Well Differentiated | 3 | 17.6% |
| | Moderately Differentiated | 7 | 41.2% |
| | Poorly Differentiated | 7 | 41.2% |
| **Level of LN** | Level I | 2 | 11.7% |
| | Level II | 7 | 41.1% |
| | Level I + II | 5 | 29.4% |
| | Level II + III | 2 | 11.7% |
| | Level IV | 1 | 5.9% |
| **Number of Nodes** | Single Node | 11 | 64.7% |
| | Multiple Nodes | 6 | 35.3% |
| **Nodal Size** | < 3 cm | 2 | 11.7% |
| | 3 – 6 cm | 12 | 70.6% |
| | > 6 cm | 3 | 17.7% |

The mean DOI was 7.23 ± 1.87 mm, ranging from 1 to 13 mm. Nodal metastasis rates increased significantly with greater DOI: 14.3% for DOI ≤5 mm, 45.5% for DOI 5.1–10 mm, and 91.7% for DOI >10 mm (p = 0.002). The mean DOI was significantly higher in the node-positive group (10.4 ± 2.7 mm) compared to the node-negative group (5.4 ± 2.3 mm; p = 0.000) (Table III).

**Table III**

*Correlation between Depth of Invasion (DOI) and Lymph Node Metastasis (n=30)*

| Variable | Category | LN Positive (n=17) | LN Negative (n=13) | p-value |
|---|---|---|---|---|
| **DOI Groups** | ≤5 mm (n=7) | 1 (14.3%) | 6 (85.7%) | 0.002* |
| | 5.1 – 10 mm (n=11) | 5 (45.5%) | 6 (54.5%) | |
| | >10 mm (n=12) | 11 (91.7%) | 1 (8.3%) | |
| **Mean DOI** | Mean ± SD (mm) | 10.4 ± 2.7 | 5.4 ± 2.3 | 0.000** |

* Chi-squared ($\chi^2$) test

** Unpaired Student's t-test

**Discussion:**

The management of the neck in patients with oral cavity squamous cell carcinoma (OCSCC) remains one of the most debated topics in head and neck oncology, primarily because regional nodal status is the single most important prognostic factor for survival. The core finding of the study shows that a deeper Depth of Invasion (DOI) significantly correlates with regional lymph node

metastasis aligning with the foundational shifts seen in the American Joint Committee on Cancer (AJCC) 8th Edition staging manual. By observing a mean DOI in node-positive patients (10.4±2.7 mm) that was nearly double that of node-negative patients (5.4±2.3 mm), the data reinforces the status of DOI as a primary driver of biological aggressiveness and regional spread. This significant statistical difference ($p<0.05$) suggests that DOI is not merely a descriptive pathomorphological feature but a functional indicator of the tumor's ability to access the lymphatic system.

Historically, clinicians and pathologists often used the terms "Tumor Thickness" and "Depth of Invasion" interchangeably; however, the AJCC 8th Edition clarified that DOI is measured from the basement membrane of the adjacent healthy epithelium to the deepest point of invasion[10]. This distinction is critical because its better accounts for the variety of growth patterns seen in the oral cavity, such as exophytic lesions that may be thick but not deep, or ulcerative lesions that are deep despite appearing flat. The results support this shift in focus toward depth. It was observed that as the tumor penetrates deeper into the extrinsic muscles of the tongue or the buccinator muscle, the likelihood of encountering the rich, high-pressure lymphatic plexus of the oral cavity increases exponentially. This biological reality is evidenced by the finding that 91.7% of tumors with a DOI >10 mm was node-positive, a figure that mirrors the high-risk profiles described in large-scale multi-institutional studies[11].

The clinical utility of these findings is most apparent when deciding whether to perform an Elective Neck Dissection (END) in patients with a clinically N0 neck. The generally accepted "20% rule"—which dictates that if the risk of occult metastasis exceeds 20%, a prophylactic neck dissection is warranted—relies heavily on reliable predictors like DOI[12]. In this cohort, tumors in the 5.1–10 mm range demonstrated a 45.5% metastasis rate, which is more than double the threshold required to justify surgical intervention. Interestingly, even the "shallowest" group (d"5 mm), a 14.3% metastasis rate was observed. While this falls slightly below the 20% rule, it suggests that DOI should not be viewed in a vacuum. Other factors, such as the "Pattern of Invasion" (POI) or the presence of lymph vascular invasion (LVI), likely play synergistic roles in facilitating nodal spread even at shallower depths[13,14].

Comparing these results to global literature reveals interesting nuances in how DOI performs across different populations. The mean DOI for metastatic cases (10.4 mm) is somewhat higher than thresholds identified in several Western cohorts, where 4 mm is often cited as the critical "tipping point" for nodal spread in early-stage OCSCC[15]. This discrepancy is likely because the study focused specifically on advanced-stage cases, where the tumors have had more time to achieve significant vertical growth. Furthermore, the high prevalence of betel quid chewing in this demographic (60%) cannot be ignored. The chronic irritation and submucous fibrosis associated with this habit may create a unique microenvironment that alters the traditional DOI-to-metastasis ratio, potentially requiring higher thresholds for prediction compared to non-chewing populations[16].

The biological rationale for the correlation we observed lies in the complex anatomical architecture of the oral cavity. As squamous cell carcinoma cells penetrate deeper into the underlying stroma, they often undergo an epithelial-mesenchymal transition (EMT), gaining the migratory capacity required to breach the basement membrane and enter the lymphatic channels[17]. Deeper penetration

typically involves the invasion of the deep lingual or facial arteries, which are surrounded by extensive, well-organized lymphatic networks designed for rapid fluid transport[18]. The study observed that poorly differentiated tumors had an 88% metastasis rate compared to only 25% in well-differentiated cases suggests that high-grade cellular features work in tandem with physical depth to facilitate regional spread.

The prognostic weight of DOI also has a direct impact on staging and, consequently, survival outcomes. The AJCC 8th Edition now upstages a T1 lesion to T2 if the DOI exceeds 5 mm, and to T3 if it exceeds 10 mm[10]. The data provides a strong validation for this upstaging; the stark jump in nodal positivity from 14.3% in the d”5 mm group to 91.7% in the >10 mm group justifies the movement across staging categories. International researchhas confirmed that a 1 mm increase in DOI significantly decreases disease-specific survival, emphasizing that DOI is a continuous variable of risk rather than just a categorical one[19,20]. This makes it an invaluable tool for personalized treatment planning, allowing surgeons to move away from “one-size-fits-all” approaches.

Despite the strength of statistical findings, this study is not without limitations. The sample size of 30 patients, while sufficient for identifying a strong correlation, may restrict the ability to calculate a truly “optimal” threshold for a broader population. Additionally, the analysis did not specifically account for the phenomenon of “skip metastases,” where tumors of the tongue might bypass Level I nodes to involve Level II or III directly—a trend sometimes observed in deep-seated lesions[21]. This research also recognizes that DOI is a physical measurement that does not capture the molecular complexity of the tumor. Future research should aim to integrate DOI with molecular markers such as p16, EGFR expression, or specific gene signatures to provide a more multidimensional and accurate predictive model for regional spread in OCSCC.

**Conclusion:**

Depth of invasion is a reliable and significant predictor of cervical lymph node metastasis in advanced (T3, T4) oral cavity squamous cell carcinoma. The risk of metastasis increases markedly once DOI exceeds 10 mm. Routine histopathological measurement of DOI should be standardized to improve staging accuracy and guide the necessity of neck management, ultimately enhancing patient outcomes.

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