The Correlation Between Depth of Invasion, and Degree of Differentiation with Cervical Lymph Node Metastasis in Oral Squamous Cell Carcinoma

Sargon Shazo[1*], Hayder Dakheel Al-Mualla[2], Abdulazeez Muayad Abdulkarem[3], Faeza Aftan Zghair[4]

[1]College of Dentistry, University of Duhok, Duhok, Iraq.
[2]Oral and Maxillofacial Surgery Department, School of Dentistry, University of Kufa, Al Najaf, Iraq.
[3]Oral and Maxillofacial Surgery Department, School of Dentistry, University of Muthana, Muthana, Iraq.
[4]Department of Histopathology, College of Medicine, Aliraqia University, Baghdad, Iraq.
*Correspondence to: Sargon Shazo (E-mail: sargon.daweed@uod.ac)

Abstract

Objective: To study the significance of Depth of invasion (DOI), and the degree of differentiation (DOD) as predictors of cervical nodal metastasis in oral squamous cell carcinoma. To determine a cut-off depth beyond which there is a high risk of cervical metastasis. To determine whether the DOI can be is alone enough as predictor for lymphatic metastasis despite the DOD.

Methods: Forty-eight patients with OSCC were included in the study 28 males and 20 females ranging between 25 to 80 years. All treated in Ghazi Al-Hariri hospital for surgical specialties in Baghdad Medical city between 2017 to 2019. Slides were examined after immunohistochemical staining. Depth of invasion was measured according to the criteria of AJCC, and the WHO system of grading was, all histopathological parameters were recorded after immunohistochemical staining.

Results: The depth of invasion ranged between 2 and 13 mm with a mean of 6.58 mm and SD of ± 2.56 mm and it was found to be a highly significant predictor for lymphatic metastasis (P-value = 0.001), the cut-off point with a significant risk of metastasis was 5 mm. The degree of differentiation was also a significant predictor for cervical metastasis (P-value = 0.024) with poorly differentiated SCC carrying the highest risk of cervical metastasis despite the depth of the tumor invasion.

Conclusion: DOI and DOD were significant histological parameters that predict the behavior of the well and moderately differentiated oral squamous cell carcinoma and hence the management of the neck in OSCC. However, the depth of invasion was not significance in cases of poorly differentiated OSCC. Therefore, we recommend that elective neck dissection should be done in cases of poorly differentiated OSCC despite the DOI.

Keywords: Metastasis, lymph, oral, carcinoma, tumor, incidence, differentiation, survival, decrease

Introduction

Oral squamous cell carcinoma OSCC is the most common head and neck cancer and it constitutes 90–95% of malignancies in oral cavity.[1,2] It is one of the 20 most common cancer in humans. The global incidence of oral squamous cell carcinoma in 2018 was estimated to be 354 864 cases with an estimated number of deaths of 177 384 people with the tongue being the most common subsite. In Iraq, the prevalence is 1.4 per 100000 persons, with 237 reported new cases in 2018 and the estimated number of deaths being146 persons.[3,4]

The prognosis of OSCC depends on many factors, like patient defense mechanism, tumor characteristics, and treatment provided. The presence of lymph node metastasis is, however, the most important prognostic factor.[5]

Cervical lymph node metastasis in OSCC is the most significant prognostic indicator and reflects an adverse step in tumorigenesis which decreases the survival to half.[6] The incidence of occult cervical metastasis in OSCC varies from 23.7% to 42%.[7,8] With this incidence of occult metastasis, there is strong evidence supporting elective neck dissection (END), however, it is clear that many patients do not have metastasis and hence no need for being subjected to additional morbidity, weather functional or esthetic, associated with neck dissection.[9]

Whether the END is advantageous to the patient or not remains an important clinical dilemma, hence, determining factors associated with the risk of lymph node metastasis may be useful for the proper selection of patients for END.[10,11]

Many clinical and histopathological factors were investigated for their relation to occult metastasis.[9,12–19]

Depth of invasion (DOI) was found to be an essential predictor for the nodal metastasis that affects the selection of treatment and eventually prognosis in oral SCC.[16,17,20] The evaluation of depth of invasion can be clinical or histopathological. Clinically palpation gives an insight into the extent of the tumor.[1] however, this procedure this methods is subjective and may lack accuracy, radiographical evaluation proved to be a valid method for measuring preoperative DOI.[21–23]

There is a difference in the predictive cut-off histopathological values for DOI as a determinant for occult metastasis,[16,18,19] this mandates further researches on this area to make a clearer cut-off point and study and what is the significance of the DOI as a risk for having cervical lymph node metastasis.

Different methods were proposed for grading of the tumor, the first and most popular one is Broder's grading system, other system evolved since then and the significance in relation to lymphatic metastasis and prognosis were evaluated.[24–26] There is a lack of consensus about the value and significance of the degree of differentiation (DOD) as a predictor or prognostic factor. We used Broder's system for tumor grading to find if it provides a clue about cervical metastasis.

In this study, we analyzed the relation between the depth of invasion and degree of differentiation as predictors for lymph node metastasis and whether the DOI alone can be determinant in the decision making of managing the neck.

Patient and Methods

Forty-Eight patients were included in this study, 28 males and 20 females ranging between 25 years to 80, all diagnosed with oral squamous cell carcinoma. The patients treated in Ghazi Al-Hariri hospital for surgical specialties in Baghdad Medical city between August 2017 to August 2019.

The pathological specimens were histopathologically examined after Hematoxylin and Eosin staining (H&E) for histopathological staging. For a more accurate evaluation of the data immunohistochemical staining was performed and the data reviewed by separate surgical pathologist

Approval for use of human subjects was obtained from Maxillofacial Surgery Review Board and Ghazi Al-Hariri Hospital for Surgical Specialties.

Exclusion Criteria

- Patients who had preoperative chemotherapy and/or radiotherapy were excluded from the study.
- Patient with only primary tumor removed were also excluded from this study
- Patient with positive deep margin that affect DOI measurements
- Patient with incomplete clinical and histopathological data

In 17 of the patients, the primary lesion was in the tongue, 17 in buccal mucosa, 10 in the lower alveolar ridge, 2 in the upper alveolar ridge, 1 in retromolar trigone, 1 in the lower lip.

The patients were clinically divided into N0 and N+ patients. Clinical and radiographical examination showed that 27 patients were N0 at the time of presentation, while 21 were N+.

Surgery

The primary lesion was resected with curative intent to attain a clear clinical margin of at least 1–1.5 cm figure (2.5). Reconstruction was done either by primary closure or by other reconstructive options e.g., skin graft, local and regional flaps Figure 1.

Sixteen patients underwent unilateral selective neck dissection USND (level I-III), 28 had USND level (I-IV), 1 unilateral radical neck dissection RND, and 3 had bilateral se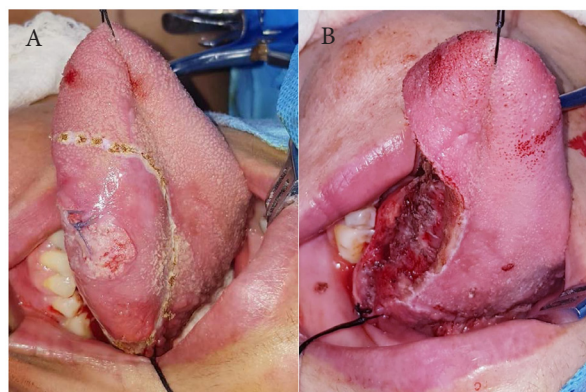lective neck dissection BSND. The specimen sent for histopathological examination was mapped with stitches and labeled then pictured and placed in 10% labeled formalin containers Figure 2.

Histopathological Examination

Hematoxylin and Eosin Staining

Hematoxylin and Eosin Sections were examined, and tumors were diagnosed and graded according to (WHO system), being well-differentiated, moderately or poorly differentiated. Depth of invasion, involvement of resection margins, lymph node metastasis, and extra-nodal extension were assessed and concluded the pathological staging.

Immunohistochemical (IHC) Staining for Cytokeratin (CK5/CK6)

To confirm the results of Hematoxylin and Eosin sections and for more accurate evaluation of the status of the depth of invasion, and pathological staging, further histological assessment was done on immunohistochemically stained sections for cytokeratin (CK5/CK6).

CK5/CK6 immune staining was performed on formalin-fixed, paraffin-embedded tissue sections, according to the manufacturer's instructions. Each block was cut at a thickness of 5μm on a microtome cutter (Leica RM2235). Sections were placed on salinized coated slides, (DAKO, UK).

Antigen retrieval steps were performed by antigen retrieval solution (ready to use). Followed by incubation with primary Anti- CK5/CK6 antibody (Rabbit monoclonal antibodies), [abcam (52B83)], Then, sections were counterstained with hematoxylin, and finally, the cover was slipped over.

Slides were scanned and scored by consultant pathologist. Positive controls of known positive tissues (Breast tissue sections and tongue squamous cell carcinoma sections) and negative controls with primary antibody replaced with TBS were run with the patient slides.

All surgical specimens had been primarily evaluated by surgical pathologists (at Ghazi Al-Hariri Hospital for Surgical Specialties – Histopathology Laboratory, and at College of Dentistry - Baghdad University). The specimens were reviewed after immunohistochemistry by a consultant surgical pathologist at the College of Medicine - Aliraqia University.
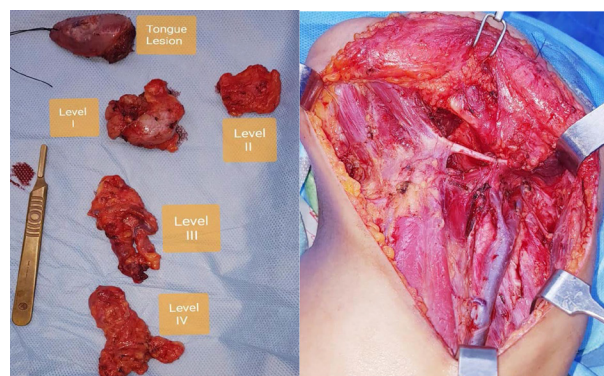

Fig. 1 **SCC of the tongue. (A) Marking the area of resection. (B) After resection.**

Fig. 2 **Selective neck dissection level (I–IV), Specimens prepared for histopathological examination.**

Evaluation of Immune Staining

Positive immunohistochemical staining for CK5/CK6 (EP24 & EP67), was expressed as diffuse golden – brown stain in the cytoplasm of tumor cells. According to DAKO scoring system Sections were categorized as negative (Staining index (SI) = 0) or as positive (Staining index (SI) > 0). From the above data and histopathological assessment, the correlation between tumor size, tumor grade, pathological stage along with the depth of invasion, were studied.

Measurement of Depth of Invasion (DOI)

Measurement of depth of tumor invasion (by millimeters) was simply done using a graded acetate sheet overlaid onto the glass slide (Figure 3).

Microscopically, it refers to the length of a perpendicular line drawn from the (horizon) of the basement membrane of the adjacent intact basement membrane to the deepest point of tumor invasion in the underlying tissues (the depth of subepithelial invasion), regardless of the nature of the lesion.

Statistical Analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. An independent t-test (two-tailed) was used to compare the depth of invasion accordingly. The Chi-square test was used to assess the association between lymph node involvement and certain information. Receiver operating characteristic (ROC) curve analysis was used for prediction of depth of invasion as a predictor for the risk of lymph node involvement. A level of P–value less than 0.05 was considered significant.

Results

The total number of study patients was 48. All of them were diagnosed with oral squamous cell carcinoma.
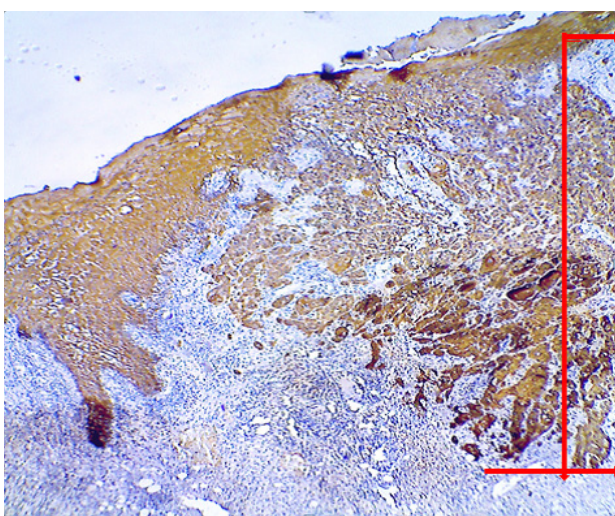

Fig. 3 **DOI is measured by a plumb line drawn from nearest normal basement membrane to the deepest point of tumor invasion.**

Age and Gender

The distribution of study patients by age and gender is shown in figures (3.1 and 3.2). Study patient's age was ranging from 25 to 84 years with a mean of 59.22 years and a standard deviation (SD) of ± 13.01 years. The highest proportion of study patients were aged between 50–69 years (58.2%) as shown in Figure 4.

Regarding gender, the proportion of males was higher than females (58.3% versus 41.7%) with a male to female ratio of 1.4:1, Figure 5.

Tumor Information

The distribution of study patients by tumor information is shown in Table 1. In this study, the most common sites of tumor were buccal mucosa and tongue (35.4% for every site). Regarding the stage of the tumor, the highest proportion of tumors was diagnosed in stage IVA (39.6%).

Concerning the depth of invasion, it was ranging from 2 to 13 mm with a mean of 6.58 mm and SD of ± 2.56 mm.

About the degree of differentiation, the highest proportion of study patients were diagnosed with well-differentiated tumor (45.8%).

Lymph Node Involvement

Out of 48 patients examined, 27 were found to have metastatic lymph nodes 56.2%. The distribution of study patients by the number of lymph nodes involved is shown in Figure 6. In this study, no lymph node involvement was noticed in 43.8% of study patients while 29.2% of them were found to be diagnosed with 1–3 lymph node involvement.
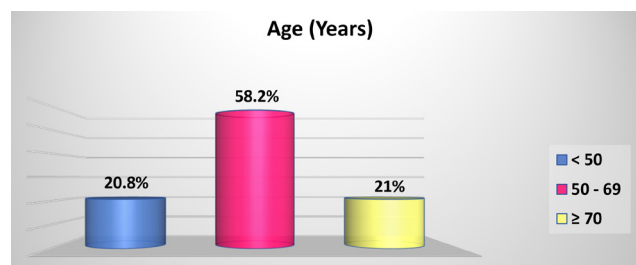

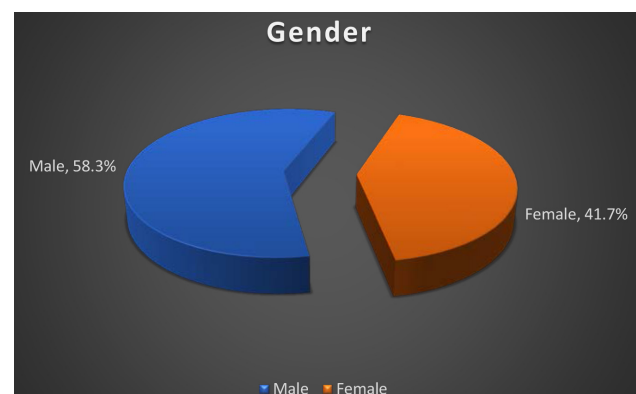Fig. 4 **Distribution of study patients by age.**

Fig. 5 **Distribution of study patients by gender.**

There was significant association between lymph node involvement and degree of differentiation as shown in Table 2. The highest prevalence of positive lymph node involvement was found in poorly differentiated tumors (83.3%) with a significant association (P = 0.024) between lymph node involvement and degree of differentiation.

Depth of Invasion (DOI)

DOI ranging from 0–5 mm had the lowest percentage of LN metastasis 31% (6 out of 19), while for the depth of 5.1–10 mm the rate was 68 % (17 out of 25), while all 4 patients with tumor depth > 10 had LN metastasis Table 2. The mean depth of invasion in pN- cases was 5.23 ± 1.84 mm while the mean for pN+ was 7.62 ± 2.58 mm. Our statistical analysis showed that the depth of invasion was a highly significant predictor for regional lymph node metastasis (P-value = 0.001) Table 3.

The comparison in mean between lymph node and depth of invasion is shown in Table 4. In this study, means of the depth of invasion in positive lymph nodes were significantly higher than that in negative lymph nodes (7.62 versus 5.23, P = 0.001).

Cut-off Point

Receiver operating characteristic (ROC) curve analysis was constructed for depth of invasion as indicators for the risk of lymph node involvement. As shown in table and Figure 7 the cut-off point of depth of invasion was 5.0 mm, hence the depth of invasion >5.0 mm is predictive for risk of lymph node involvement as a large significant area under the curve (AUC = 76.7%) indicating significant association between higher depth of invasion and risk of lymph node involvement.

Table 5 illustrates the diagnostic accuracy of the cut-off point of DOI.

The comparison in mean depth of invasion by lymph node involvement according to the degree of differentiation is shown in Table 6. We noticed that means the depth of invasion in patients with well and moderately differentiated tumors were significantly higher those who had positive lymph node involvement (7.25 versus 5.0 mm, P = 0.039; and 8.44 versus 5.8 mm, P = 0.028 respectively).

Depth of invasion was not significant (P = 0.42) in patients with poorly differentiated tumors between positive and negative lymph node involvement.

Discussion

Oral squamous cell carcinoma is the debilitating disease with a global prevalence of 12 per 100000.[4] Despite the efforts to improve the outcome of treatment, the overall improvement of the survival rate has not improved.

Regional cervical LN metastases are treated by neck dissection,[27] however, the treatment of the patients with a clinically negative neck is still controversial.[28] Proponent for watch and see policy in early SCC states that there is no need to

Table 1. **Distribution of study patients by tumor information**

| Tumor information	No. (n = 48)	Percentage (%)
Site of tumor		
Buccal mucosa	17	35.4
Tongue	17	35.4
Lower Alveolar Ridge	10	20.8
Upper Alveolar Ridge	2	4.2
Lower Lip	1	2.1
Retromolar Trigone	1	2.1
Stage of tumor		
I	3	6.3
II	10	20.7
III	13	27.1
IVA	19	39.6
IVB	3	6.3
Depth of invasion in mm		
0–5	19	39.6
5.1–10	25	52.1
>10	4	8.3
Degree of Differentiation		
Well Differentiated	22	45.8
Moderately Differentiated	14	29.2
Poorly Differentiated	12	25.0
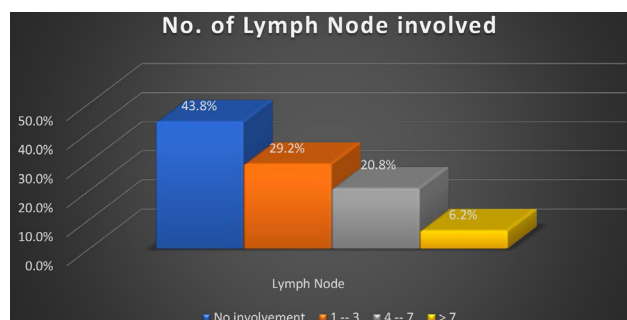
Fig. 6 **Distribution of study patients by the number of lymph node involved.**

Table 2. **Association between lymph node involvement and DOD**

Degree of differentiation	Lymph node involvement		Total (%) n = 48	P-value
	Negative (%) n = 21	Positive (%) n = 27		
Well differentiated	14 (63.6)	8 (36.4)	22 (45.8)	0.024
Moderately differentiated	5 (35.7)	9 (64.3)	14 (29.2)	
Poorly differentiated	2 (16.7)	10 (83.3)	12 (25.0)	

subject the patient to the morbidity of END considering the high percentage of pN-, in addition to the destruction of the natural barrier of the disease.[29] The study of predictive factors is of extreme importance, as it optimizes the outcome of treatment, and hence the prognosis. This study intended to investigate the predictive value of two parameter in the primary lesion in OSCC and whether the depth of the lesion is solely responsible for early regional metastasis.

Immunohistochemistry (IHC) was found valuable in detecting micrometastases in histopathologically negative node Yoshida et al.,[30] observed micrometastases in 14 out of 24 pN nodes (58%) after IHC, therefore, and for more accurate results we depended on results after immunohistochemistry to measure the depth of invasion and degree of differentiation.

Lymph Node Involvement

Regional lymph node metastasis of the neck is one of the major prognostic factors in OSCC, patients with neck metastasis have reduced survival rate by half.

Of the 48 patients included in our study 27 of them were presented with clinically negative neck lymph nodes. Histopathologically, the rate of occult metastasis in our study was 22.2% (6/27) which is closely related to the rate of occult metastasis published in the literature.[9,13,18,31]

A total of 824 lymph nodes were examined ranging between 7 to 31 node per case, with a mean of 17.12 and standard deviation (SD) of ± 5.67, the number of involved lymph nodes was 110 lymph nodes (13.3%).

Level I and II were the most involved by regional metastasis 56.25% (27/48) followed by level III 23% (11/48), and finally, level IV was the least involved 16.6% (8/48).

Interestingly, there was no evidence of skip metastasis to level III and IV in all cases examined including those with tongue SCC, and these levels were involved only when the previous level showed metastatic nodes.

Byers et al.[32] were among the first to describe skip metastasis and he reported a 15.8% rate of skip metastasis and they recommended routine inclusion of level IV in neck dissection. Woolgar[33,34] one of the supporters of the theory of skip metastasis also recommended including level IV of the neck in all cases of tongue SCC despite the neck status.

A systematic review for assessing the rate of skip metastasis to level IV conducted by Warshavsky et al.[35] found that the rate of skip metastasis was very low (0.5%) and even in cases of where level I through III were involved the rate of level IV involvement was not significant and therefore he recommended supraomohyoid neck dissection SOHND is adequate for patient with clinically negative neck.

The highest prevalence of positive lymph node involvement was found in poorly differentiated tumors (83.3%) with a significant association ($P = 0.024$) between lymph node involvement and degree of differentiation.

Depth of Invasion

Depth of invasion has been studied over the last decades for its significance as a predictor for lymph node metastasis and

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Table 3. **Percentage of nodal involvement according to DOI**

Depth of invasion in mm	No of patients	N+ (%)
0–5	19	6 (31)
5.1–10	25	17 (68)
>10	4	4 (100)

Table 4. **Comparison of the mean DOI with to lymph node involvement**

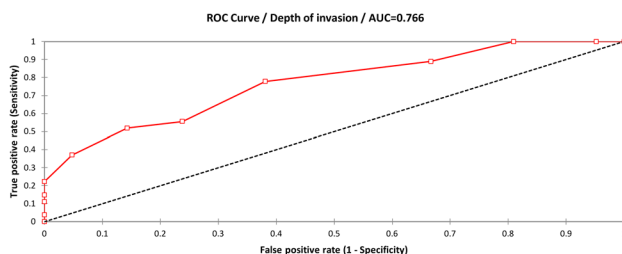| Depth of invasion (mm)	Lymph node involvement		P-value
	Negative Mean ± SD	Positive Mean ± SD	
	5.23 ± 1.84	7.62 ± 2.58	0.001

Fig. 7 **ROC curve for DOI as a predictor of lymph node involvement.**

Table 5. **Diagnostic accuracy of DOI for risk of lymph node involvement**

Depth of invasion (mm)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	5.0	77.8%	61.9%	72.4%	68.4%	70.8%

Table 6. **The significance of DOI with different stages of DOD on LN metastasis**

Degree of differentiation	Depth of invasion according to lymph node involvement (mm)		P-value
	Negative LN Mean ± SD	Positive LN Mean ± SD	
Well differentiated	5.0 ± 1.83	7.25 ± 2.96	0.039
Moderately differentiated	5.8 ± 1.48	8.44 ± 2.45	0.028
Poorly differentiated	5.5 ± 3.53	7.2 ± 2.48	0.42

prognostic factor. The latest edition of the American Joint Committee Cancer AJCC manual included the DOI as part of the TNM staging system in the T section.[36]

In our study, the depth of invasion was measured according to the proposed method of the for AJCC.[3] The DOI ranged from 2–13 mm with a mean of 6.58 mm and SD of ± 2.56 mm, we noticed an increase in the rate of metastasis with increased depth of more than 5 mm.

DOI ranging from 0–5 mm had the lowest percentage of LN metastasis 31% (6 out of 19), while for the depth of 5.1–10 mm the rate was 68 % (17 out of 25). statistical analysis demonstrated significant predictive value of DOI (*P*-value = 0.001) Table 4.

When Zhang et al.[37] studied the histological characteristic of OSCC at different depth of the same tumor (surface and central depth, and deep infiltration), they found the features worsens at the deepest point (tumor frontier), this front carries the highest ability of invasion and hence metastasis. This can be considered a logical explanation for the invasiveness of the tumor as it becomes deeper.

Melcher et al.[38] in his retrospective study of 212 cases with pT1cN0, found that the depth of invasion was significant predictor for occult neck metastasis with the metastasis proportionally related to increased DOI. They determined an optimal cut-off point of 4.59 mm as a predictor for nodal status and recommended 4 mm DOI as an indication for END.

O-charoenrat et al.[39] as well, found that patients with depth < 5 mm had 95% five-year survival rate compared with 30% for patients with DOI >5 mm. Faisal et al.,[16] as well reported a significant relationship between the depth of invasion and LN metastasis, he found that tumors with depth >10 mm had the highest rate of LN metastasis, however, he didn't determine a cut-off point.

There were disparities between our results and other studies regarding the optimum cut-off point for DOI. Warburton et al.,[40] determined an optimum cut-off point of 2.2 mm to optimize sensitivity and specificity (87.5 % and 78.9%) respectively, which is different from our results, though, when analyzing the data and methodology, we found that they measured the thickness, as defined by Moore, instead of true depth, therefore the ulcer was measured from the base to the deepest point, giving smaller cut-off value.

Hoşal et al.,[41] also, stated that the tumor depth of the tumor was a significant predictor but, he concluded that tumor deeper than 9 mm is an indication for neck dissection. Instead of grouping the data of depth into closer numbers (0, 5, 10 mm), Hoşal and colleagues grouped the patients into those with depth <9 mm and >9 mm and which led, in our opinion, to these results.

The fact that there is a disparity between the values of cut-offs might be attributed to differences in methodology, the procedure of measurement according to the researcher's definition of DOI, and the number of slices examined per case.

Degree of Differentiation

The degree of differentiation of cancer is a qualitative process that reflects the extent of tumor resemblance with normal tissue architecture at that site.[1] It has the disadvantage of being subjective and to some extent pathologist dependent.[2]

In this study, the WHO grading system was adopted and patients were grouped into well-differentiated, moderately differentiated, and poorly differentiated depending on the

criteria set by Broder,[24,42] depending on the certain histologic features like the degree of keratinization, cellular and nuclear pleomorphism, and mitotic activity.

Opponents of this system state that it is of limited prognostic and predictive value because it depends on certain histologic features that are highly variable in the same tumor.[43] However, Odell[44] found a highly significant correlation between Broder's grading system and metastasis. Akhter[12] as well, found that both Broder and Anneroth systems were significantly related to lymphatic metastasis (P-value = 0.002, 0.012 respectively).

The majority of the patients included in our study had well-differentiated SCC (n = 22) 45.8%, only 8 of them had nodal involvement (36.4%), While 14 patients (29.2%) had moderately differentiated tumors with 9 having positive node (64.3%), finally those with poorly differentiated tumors were 12 (25%) with 10 of them having regional lymph node metastasis (83.3%).

Bivariant statistical analysis showed a significant relationship between the grade of the tumor and the nodal status (*P*-value = 0.024) Table 2.

The increase in the mitotic figure of cancer cell, the abundance of cancer progenitor cells that have high ability to produce tumor-specific circulation, as well as increased expression of certain oncogenes like epidermal growth factor receptor EGFR; explain the increase in metastatic rate with poorer differentiation.

Our results were in line with those of Kavabata et al.[45] who reported a significant relationship between the grade of the tumor using WHO system and possibility of late lymph node metastasis (LLNM), other studies results were in consensus with ours especially those of Li et al.,[14] Soni et al.,[46] Faisal et al.,[16] Haksever et al.,[29] and Goerkem.[47]

In contrary to our findings Chaudhary et al.,[9] failed to find any relation between the degree of differentiation and lymph node metastasis and recommended END for all cases of OSCC. Neena et al.,[43] and Khalili and Alyani,[15] also concluded that the degree of differentiation is not a reliable histologic parameter for the risk of metastasis due to the subjective nature some grading systems and the heterogenicity of the tumor cells, and that further search for other predictor must continue.

We noticed from our results that patients with poorly differentiated SCC had the highest rate of nodal metastasis despite the depth of invasion. A question was raised about the significance of depth as a predictor in different states of differentiation.

We tested our observation statistically, interestingly the DOI was not a significant predictor in cases of poorly differentiated SCC (P-value = 0.42), however, it was significant in well and moderately differentiated SCC (P-value = 0.039, 0.028 respectively) Table 5.

Two possible explanations for this finding can be given:

- Because of the heterogeneous nature of squamous cell carcinoma, various stages of differentiation can be seen in different cuts of the same tumor specimen, and because the WHO grading system is subjective, this may lead to different interpretations between pathologists and their experience.
- At the molecular level, various oncogenes are involved in the development, invasion, and metastasis of the tumor. The most widely studied is the

epidermal growth factor receptor EGFR, it binds to epidermal growth factor EGF which regulates cell growth.[48] EGFR is aberrantly overexpressed in 92% of the cases of head and neck squamous cell carcinoma HNSCC and with increase expression in advanced stage and poorly differentiated SCC.[49] O-Charoenrat et al. found an association between EGFR activation and matrix metalloproteinase (MMP) leading to the belief in its role in invasion aand metastasis.[50]

Conflict of Interest

The authors have no conflict of interest with regard to this manuscript. ■

References

1. Shah J. Oral Cavity. In: Shah JP, Patel SG, Singh B, Wong R, editors. Jatin Shah's Head and Neck Surgery and Oncology. fifth edit. Elsevier; 2019. p. 245–97.
2. Neville BW. Epithelial Pathology. In: Neville BW, Damm DD, Allen CM, Chi AC, editors. Oral and Maxillofacial Pathology. 4th ed. Elsevier Inc.; 2016. p. 331–421.
3. Ridge JA, Lydiatt WM, Patel SG, Glastonbury CM, Brandwein-Gensler M, Ghossein RA, et al. Lip and Oral Cavity. In: Amin M, editor. AJCC Cancer Staging Manual. eighth. Cambridge: Springer; 2017. p. 79–94.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
5. Leemans CR, Tiwari R, Nauta JPJ, Van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck carcinoma. Cancer. 1993;71(2):452–6.
6. Shah JP. cervical lymph nodes. In: Shah J, Patel SG, Singh G, Wong R, editors. Jatin Shah's Head and Neck Surgery and Oncology. fifth edit. Elsevier; 2019. p. 441–88.
7. Okamoto M, Nishimine M, Kishi M, Kirita T, Sugimura M, Nakamura M, et al. Prediction of delayed neck metastasis in patients with stage I/II squamous cell carcinoma of the tongue. J Oral Pathol Med. 2002;31(4):227–33.
8. Khafif RA, Gelbfish GA, Tepper P, Attie JN. Elective radical neck dissection in epidermoid cancer of the head and neck. A retrospective analysis of 853 cases of mouth, pharynx, and larynx cancer. Cancer. 1991;67(1):67–71.
9. Chaudhary N, Verma R, Agarwal U, Gupta S, Jaitly S. Incidence of occult metastasis in clinically N0 oral tongue squamous cell carcinoma and its association with tumor staging, thickness, and differentiation. J Head Neck Physicians Surg. 2018;5(2):75.
10. Kowalski LP. Results of Salvage Treatment of the Neck in Patients With Oral Cancer. JAMA Otolaryngol Neck Surg [Internet]. 2002 Jan 1;128(1):58–62. Available from: https://doi.org/10.1001/archotol.128.1.58
11. Capote A, Escorial V, Muñoz-Guerra MF, Rodríguez-Campo FJ, Gamallo C, Naval L. Elective neck dissection in early-stage oral squamous cell carcinoma—does it influence recurrence and survival? Head Neck [Internet]. 2007 Jan 1;29(1):3–11. Available from: https://doi.org/10.1002/hed.20482
12. Akhter M, Rahman Q, Hossain S, Molla M. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011;15(2):168.
13. Jacob TE, Malathi N, Rajan ST, Augustine D, Manish N, Patil S. Histopathological parameters predicting occult nodal metastases in tongue carcinoma cases: An Indian perspective. J Contemp Dent Pract. 2016;17(1):70–7.
14. Li Y, Liu K, Ke Y, Zeng Y, Chen M, Li W, et al. Risk Factors Analysis of Pathologically Confirmed Cervical Lymph Nodes Metastasis in Oral Squamous Cell Carcinoma Patients with Clinically Negative Cervical Lymph Node: Results from a Cancer Center of Central China. J Cancer. 2019;10(13):3062–9.
15. Khalili M, Alyani Z. Clinicopatholgic Parameters Associated with Regional Lymph Node Metastasis in Oral Squamous Cell Carcinoma: A Retrospective Study. Iran J Pathol. 2008;3(3):140–6.
16. Faisal M, Bakar MA, Sarwar A, Adeel M, Batool F, Malik KI, et al. Depth of invasion (DOI) as a predictor of cervical nodal metastasis and local recurrence in early stage squamous cell carcinoma of oral tongue (ESSCOT). PLoS One. 2018;13(8):1–10.
17. Fukano H, Matsuura H, Hasegawa Y, Nakamura S. Depth of invasion as a predictive factor for cervical lymph node metastasis in tongue carcinoma. Head Neck. 1996;19(3):205–10.
18. Imai T, Satoh I, Matsumoto K, Asada Y, Yamazaki T, Morita S, et al. Retrospective observational study of occult cervical lymph-node metastasis in T1N0 tongue cancer. Jpn J Clin Oncol. 2017;47(2):130–6.
19. Kane S V., Gupta M, Kakade AC, D'Cruz A. Depth of invasion is the most significant histological predictor of subclinical cervical lymph node metastasis in early squamous carcinomas of the oral cavity. Eur J Surg Oncol. 2006;32(7):795–803.
20. Shah J. Head and neck. In: Shah JP, Patel SG, Singh B, Wong R, editors. Jatin Shah's Head and Neck Surgery and Oncology. fifth edit. Elsevier; 2019. p. 245–97.
21. Klein Nulent TJW, Noorlag R, Van Cann EM, Pameijer FA, Willems SM, Yesuratnam A, et al. Intraoral ultrasonography to measure tumor thickness of oral cancer: A systematic review and meta-analysis. Oral Oncol [Internet]. 2018;77(November 2017):29–36. Available from: https://doi.org/10.1016/j.oraloncology.2017.12.007
22. Mao MH, Wang S, Feng Z en, Li JZ, Li H, Qin L zheng, et al. Accuracy of magnetic resonance imaging in evaluating the depth of invasion of tongue cancer. A prospective cohort study. Oral Oncol [Internet]. 2019;91(October 2018):79–84. Available from: https://doi.org/10.1016/j.oraloncology.2019.01.021
23. Park JO, Jung SL, Joo YH, Jung CK, Cho KJ, Kim MS. Diagnostic accuracy of magnetic resonance imaging (MRI) in the assessment of tumor invasion depth in oral/oropharyngeal cancer. Oral Oncol [Internet]. 2011;47(5):381–6. Available from: http://dx.doi.org/10.1016/j.oraloncology.2011.03.012
24. Anneroth G, Batsakis J, Luna M. Review ofthe literature and a recommended system of malignancy grading in oral squamous cell carcinomas GORAN. 1986;3(8):229–49.
25. Lund C, Sogaard H, Elbrond O, Jorgensen K, Andersen AP. Epidermoid carcinoma of the lip. Histologic grading in the clinical evaluation. Acta Radiol Ther Phys Biol. 1975;14(5):465–74.
26. Bryne M, Stene T, Koppang HS, Lilleng R, Dabelsteen E, Bang G. New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. J Oral Pathol Med. 1989;
27. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Head and Neck Cancers [Internet]. 2019. Available from: www.NCCN.org
28. Yii NW, Patel SG, Rhys-Evans PH, Breach NM. Management of the N0 neck in early cancer of the oral tongue. Clin Otolaryngol Allied Sci. 1999;24(1):75–9.
29. Haksever M, Tunçel Ü, Kürkçüoglu ŞS, Uyar M, Genç Ö, Irkkan Ç, et al. The effects of tumor size, degree of differentiation, and depth of invasion on the risk of neck node metastasis in squamous cell carcinoma of the oral cavity. Ear, Nose Throat J. 2012;91(3):130–5.
30. Yoshida K, Kashima K, Suenaga S, Nomi N, Shuto J, Suzuki M. Immunohistochemical detection of cervical lymph node micrometastases from T2N0 tongue cancer. Acta Otolaryngol. 2005;125(6):654–8.
31. Mair MD, Shetty R, Nair D, Mathur Y, Nair S, Deshmukh A, et al. Depth of invasion, size and number of metastatic nodes predicts extracapsular spread in early oral cancers with occult metastases. Oral Oncol. 2018;81(April):95–9.
32. Byers R, Weber R, Andrews T, McGill D, Kare R, Wolf P. Frequency and therapeutic implications of "skip metastasis" in the neck from squamous carcinoma of the oral tongue. Head Neck. 1997;19(1):14–9.
33. Woolgar JA. Histological distribution of cervical lymph node metastases from intraoral/oropharyngeal squamous cell carcinomas. Br J Oral Maxillofac Surg. 1999;37(3):175–80.
34. Woolgar JA. Detailed topography of cervical lymph-node metastases from oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 1997;26(1):3–9.
35. Warshavsky A, Rosen R, Nard-Carmel N, Abu-Ghanem S, Oestreicher-Kedem Y, Abergel A, et al. Assessment of the rate of skip metastasis to neck level iv

in patients with clinically node-negative neck oral cavity squamous cell carcinoma: A systematic review and meta-Analysis. JAMA Otolaryngol - Head Neck Surg. 2019;145(6):542–8.

36. Edition E. 8th AJCC Cancer Staging Form Supplement 6-2018 update. AJCC Cancer Staging Manual, 8th Ed [Internet]. 2018;(Junio):192–4. Available from: https://cancerstaging.org/references-tools/deskreferences/Documents/AJCC Cancer Staging Form Supplement.pdf

37. Zhang Q, Chen K, Qui J. Clinics in Surgery Difference of WHO Pathological Grading among Different Depths in Oral Squamous Cell Carcinoma and Its Clinical. Clin Surg. 2017;2:2017.

38. Melchers LJ, Schuuring E, Van Dijk BAC, De Bock GH, Witjes MJH, Van Der Laan BFAM, et al. Tumour infiltration depth ≥4 mm is an indication for an elective neck dissection in pT1cN0 oral squamous cell carcinoma. Oral Oncol. 2012;48(4):337–42.

39. O-charoenrat P, Pillai G, Patel S, Fisher C, Archer D, Eccles S, et al. Tumour thickness predicts cervical nodal metastases and survival in early oral tongue cancer. Oral Oncol. 2003;39(4):386–90.

40. Warburton G, Nikitakis NG, Roberson P, Marinos NJ, Wu T, Sauk JJ, et al. Histopathological and Lymphangiogenic Parameters in Relation to Lymph Node Metastasis in Early Stage Oral Squamous Cell Carcinoma. J Oral Maxillofac Surg. 2007;65(3):475–84.

41. Hoşal AŞ, Ünal ÖF, Ayhan A. Possible prognostic value of histopathologic parameters in patients with carcinoma of the oral tongue. Eur Arch Oto-Rhino-Laryngology. 1998;255(4):216–9.

42. Sapp JP. Epithelial disorder. In: Sapp JP, Eersole LR, Wysocki GP, editors. Contemporary Oral and Maxillofacial Pathology. second. Mosby; 2004. p. 164–2016.

43. Neena P D, Siddarth S, Keyuri P, Munira J. Histological grading of oral cancer: a comparison of different systems and their relation to lymph node metastasis. 2002;2(1):136–42.

44. Odell EW, Jani P, Sherriff M, Ahluwalia SM, Hibbert J, Levison DA, et al. The prognostic value of individual histologic grading parameters in small lingual squamous cell carcinomas. The importance of the pattern of invasion. Cancer. 1994;74(3):789–94.

45. Kavabata NK, Caly DN, Ching TH, Gonçalves AJ, Kowalski LP, Cernea CR. Predictive factors for late cervical metastasis in stage I and II squamous cell carcinoma of the lip. Eur Arch Oto-Rhino-Laryngology. 2019;276(7):2047–53.

46. Soni S, Soni TP, Patni N. Association between nodal metastasis and histopathological factors in postoperative gingivo-buccal complex squamous cell carcinoma: A Retrospective Study. Gulf J Oncolog. 2019;(29):66–71.

47. Goerkem M, Braun J, Stoeckli SJ. Evaluation of clinical and histomorphological parameters as potential predictors of occult metastases in sentinel lymph nodes of early squamous cell carcinoma of the oral cavity. Ann Surg Oncol. 2010;17(2):527–35.

48. Dhanda J, Shaw RJ. The Molecular Biology of Head and Neck Cancer. In: Brennan P, Schliephake H, Ghali GE, Cascarini L, editors. Maxillofacial Surgery. 3rd ed. Elsevier; 2017. p. 243–56.

49. Ward BB, Helman JI. Squamous Cell Carcinoma of the Oral and Maxillofacial Region. In: Fonseca RJ, editor. Oral and Maxillofacial Surgery. 3rd ed. Elsevier; 2018. p. 670–89.

50. O-Charoenrat P, Modjtahedi H, Rhys-Evans P, Court WJ, Box GM, Eccles SA. Epidermal growth factor-like ligands differentially up-regulate matrix metalloproteinase 9 in head and neck squamous carcinoma cells. Cancer Res. 2000;60(4):1121–8.