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

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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 100 000 persons, with 237 reported new cases in 2018 and the estimated number of deaths being 146 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.8

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.9,10 Many clinical and histopathological factors were investigated for their relation to occult metastasis.11–19

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.20–22 The evaluation of depth of invasion can be clinical or histopathological. Clinically palpation gives an insight into the extent of the tumor,23 however, this procedure this methods is subjective and may lack accuracy, radiographical evaluation proved to be a valid method for measuring preoperative DOI.24–26

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.
- Patients with only primary tumor removed were also excluded from this study
- Patients with positive deep margin that affect DOI measurements
- Patients 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 selective 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.
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 sub-epithelial 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.

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).

Concerning 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.
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.

### Discussion

Oral squamous cell carcinoma is the debilitating disease with a global prevalence of 12 per 100000. 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, however, the treatment of the patients with a clinically negative neck is still controversial. Proponent for watch and see policy in early SCC states that there is no need to...
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.\textsuperscript{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.,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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.\textsuperscript{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...
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.\(^6\)

In our study, the depth of invasion was measured according to the proposed method of the for AJCC.\(^1\) 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.\(^8\) 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.\(^8\) 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.\(^9\) 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.,\(^10\) 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.,\(^11\) 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.\(^12\) as well, 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,\(^13,14\) 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.\(^15\) However, Odell\(^16\) found a highly significant correlation between Broder’s grading system and metastasis. Akhter\(^17\) as well, found that both Broder and Amneroth 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.,\(^18\) 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.,\(^19\) Soni et al.,\(^20\) Faisal et al.,\(^21\) Hakeseyer et al.,\(^22\) and Goerkem.\(^23\)

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.,\(^24\) and Khalili and Alyani,\(^25\) 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 heterogeneity 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.46 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 and metastasis.50

Conflict of Interest

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

References


