

ORIGINAL ARTICLE: Depth of Invasion as tumor indicator in cervical lymph node metastasis in Oral Squamous Cell Carcinoma- A Cross Sectional Study

Comment [i-1]: Better to mention the site and type of study.

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Comment [i-3]: Try not keeping abbreviation in the title itself.

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ABSTRACT

Squamous cell carcinoma of oral cavity is the sixth common cancer of the world and a major cause of malignancy around the world. The best prognostic indicators for the occult lymph node metastasis are tumor depth of invasion and lymph node biopsies.

SAMPLE ABSTRACT:

Aim: This study was conducted to assess the association of tumor depth of invasion and lymph nodes metastasis in oral cancer diagnosis at its initial stage.

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Methodology: A Cross sectional study was conducted at the Ziauddin hospital, North nazimabad after Ethical Review Committee approval (Reference Code: 0330618FAOM) from January 2021 to January 2022. Total 140 biopsied surgical resection specimen slides were included. The slides were evaluated by 3 investigators.

Comment [i-7]: Add date of study here.

Results: The mean age of 140 OSCC was found to be 46.87 ± 12.25 years. 80.7% of study population comprised of males. 50% of patients had T4 stage. Moderately differentiated grade (89.3%) was the most predominant grade observed in this study. On the basis of Depth of invasion the patients were divided into 2 groups. Group one with Depth of invasion upto 4 mm and second group with Depth of invasion >4 mm. Depth of invasion above 4 mm was found in 82.1% and without nodal metastasis (40.7%). The mean tumor size was found to be 4.36 ± 2.433 mm and tumor thickness was 1.95 ± 1.48 mm. and the most common anatomical site was found to be buccal mucosa (73.6%). There is a significant association of depth of invasion with Tumor stage (P value 0.001), lymph node involvement (P value 0.023) and thickness of tumor (P value 0.002) respectively.

Comment [i-8]: Both have already come in methodology. So no need of repetition.

Comment [F9]: Done

Comment [i-10]: Never start sentence with number. Holds true for all afterwards.

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Comment [i-14]: spelling

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Conclusion: The tumor depth of invasion was found to be an important variable that can be related with metastasis in Oral cancer as our study showed its significant association with lymph node metastasis. OSCC patients with a Depth of invasion >4 mm had increased the probability of lymph node metastasis than those having depth of invasion upto 4 mm.

Keywords: Depth of Invasion, Oral Squamous Cell Carcinoma, Lymph node metastasis, Tumor Thickness

INTRODUCTION

Oral squamous cell carcinoma develops from epithelial lining of the oral cavity. It is the sixth common cancer of the world and a major source of malignancy around the world (1-3). It comprises 2 to 5 % of overall malignancies in the West but in the less developed countries of Asia it is around 40 % greatly increasing the burden of disease (4).

Early oral cancer detection and diagnosis leads to a better prognosis, increases the chances of patient survival and plays a significant role in successful clinical treatment outcome. Delayed detection may lead to treatment failure and death (5). The current available treatment options for the oral cancers are not satisfactory, and the survival rate from past 5 years has not improved significantly over the past two-decades (5-8).

Lymph node metastasis is the most common source of oral cancer metastasis. Surgical resection of tumour with neck dissection is the most important treatment of choice for the patient survival in oral cancer.

Elective neck dissection gives information of pathological neck nodes involvement, hence remove undetectable cancerous cells in the lymphatic vessels. The 5-year survival rate for patients of oral squamous cell carcinomas with lymph node metastasis is upto 50%. (9-11).

According to American Joint Committee on Cancer 8th edition Depth of invasion is measured as "distance from horizon of basement membrane of adjacent squamous mucosa to deepest point of tumor in perpendicular direction through a plumbline" (12).

Now-a-days, the best prognostic indicators for the occult lymph node metastasis are Depth of Invasion and lymph node biopsies. Sentinel lymph node biopsies are highly accurate for identification of the lymph nodes metastasis. Whereas, In early oral squamous cell carcinoma patients, Depth Of Invasion is used as a marker for elective neck dissection. (10).

The purpose of this study was to assess the association of Depth of invasion and the risk of occult lymph nodes metastasis in the early oral cancer diagnosis.

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2. MATERIAL AND METHODS:

A Cross sectional study was conducted at Ziauddin hospital, North nazimabad after the Ziauddin University Ethical Review Committee approval (Reference Code: 0330618FAOM). The diagnostic histological slides of 140 patients with OSCC managed between January 2021 and January 2022 from the Ziauddin Hospital North Nazimabad histopathology department were collected from the hospitals' archives. Informed written consent was obtained from patients/ guardian visiting ziauddin hospital north nazimabad.

A total of 140 paraffin embedded foramlin fixed Oral Squamous Cell Carcinoma tissue slides were included. Patients with any other cancer of oral cavity were excluded from study by asking the patients/guardian visiting Ziauddin hospital north nazimabad. 2 investigators (F.A. and S.F.) evaluated H&E slides by light microscopy which were further reviewed by a senior pathologist (S.B). A pilot study of 20 cases was conducted to standardize the evaluation criteria for all 3 investigators. At the time of the evaluation all 3 investigators were blinded to the clinicopathologic data.

All Patients file records were recorded which includes age of the patient, gender, tumor site, tumor staging (Tumour Node Metastasis), the Depth of invasion, Lymph node involvement, tumour differentiation grades, perineural invasion and lymphovascular invasion.

Comment [i-26]: Please rephrase

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Comment [i-28]: What about consent from the patient and permission from the department?

Comment [F29]: When ethical approval was obtained from ziauddin hospital it means histopathology department has given us permission to perform the research.

Comment [i-30]: What was the kappa value?

Comment [F31]: Interclass correlation was performed to check interexaminer reliability, since our measuring scale was continuous. ICC=0.7
10% of total sample size.

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Measurement of Depth of Invasion: The hematoxylin and eosin slides were used to measure depth of invasion which was measured in accordance with AJCC 8th edition as “a plumb-line from the basal membrane of the closest normal adjacent mucosa to the deepest point of invasion”. A cut-off value of 4mm was used for depth of invasion.(10, 11).

3. RESULTS AND DISCUSSION

The present study included 140 OSCC patient with mean age of 46.87 ± 12.25 years. 80.7% of patients were males. the most common anatomical site was found to be buccal mucosa (73.6%). The mean tumor size was 4.36 ± 2.433 mm and tumor thickness was 1.95 ± 1.48 mm. the most common grade of OSCC was found to be moderately differentiated grade present in 89.3% of patients. 50% of patients had T4 stage followed by 25% of patients with T2 Stage. Lymph node metastasis was present in 59.3% of cases. on the basis of DOI the patients were divided into 2 groups. One with DOI upto 4 mm and other group with DOI >4 mm. depth of invasion up to 4mm was present in 17.9% of patients whereas above 4 mm was found in 82.1%.

the association of depth of invasion with clinicopathological parameters was assessed. T Stage, Nodal status and Tumour thickness showed significant association with depth of invasion with P values 0.001, 0.023 and 0.002 respectively.

The association of age, gender, anatomical site, grade, lymphovascular and perineural invasion with depth of invasion were non significant with p values 0.091, 0.11, 0.075, 0.511, 0.148 and 0.422 respectively.

Presence of Lymph node metastasis, blood vessels and perineural involvement and grading are important key factors in the prognosis of OSCC patient. The clinical significance and importance of the histopathological parameters are determined by clinical TNM classification.(9, 13). However, discrepancy still exists. Smaller tumor size in early oral cancer diagnosed cases have worst prognosis. Which indicates that the tumor aggressiveness & tumor biological environment does not depend upon TNM Staging(14).

Literature reported the Tumour Depth of Invasion as a predictor of occult lymph node metastasis, and it is used as an independent prognostic factor to decide on End Node Dissection in early Oral squamous cell carcinoma. The optimal cutt of point of depth of invasion was taken 4mm. The histomorphometric parameter of the tumor are best recognized after the surgical resection of the tumour. therefore treatment planned of such cancer patients depend upon the surgical resected tumour specimens results(10, 15).

Numerous studies reported tumor depth of invasion and tumor thickness are not the same. “Depth of invasion” means the extent of cancerous lesion growth into the tissue beneath an epithelial surface, Where as tumor thickness is associated with whole tumor mass.(11,12, 16,18).

Literature suggested that the Depth of invasion of tumor as a occult lymph node metastasis predictor, In early oral cancer diagnosis END is used as a tumour predictor. For indurated tumours, The DOI is considered a better prognostic factor than Tumour Thickness. The 8th edition of AJCC guideline clearly stated the Depth of invasion definition. (i.e., the distance between the basement membrane of normal mucosa and the lowest deep point of tumor invasion. Our study showed with depth of invasion >4mm raised significantly the risk of nodal involvement. some studies do not confirm cut off value of 4mm. The difference in values of depth of invasion was due to poor sampling techniques, and variation in thickness was due to variation in measuring techniques. Desparity exist in literature number of studies showed large variance. Some studied reported cut off value of <5mm DOI, Some showed cut off value of > 5mm. However, Some showed 7.25mm cut off. However, Some reported 4mm cut off. Some did not specify any cut off value for DOI, Some showed DOI value cutt off varied from different subsites for tongue 2mm, floor of mouth 3mm. The reason for lower cut off value for tongue as compared to other subsite was the higher risk of lymph node metastasis due to its rich vascular and lymphatic supply and floor of the mouth.(10,17-21).

Literature revealed there was a significant correlation between the tumor thickness and cancer metastasis at an early stage. Studies showed a strong association co-exist between the tumor depth of invasion and nodal status involvement, Which were concurrent with our study. Their study showed a depth of invasion of 4 mm to be a valuable cut-off for the occurrence of lymph node metastasis. They have recommended that oral cancers with 4mm DOI are at higher risk of metastasis and should be selected for elective nodal dissection(22). But no significant association were reported with pathological tumor size and tumor grade in tumor metastasis. Despite our study showed significant association of tumor size and tumor grade. The reason for that was tumor thickness became more significantly co-related with larger tumor size (T2–T4) which were concurrent with other studies. In our study, we had OSCC patients with tumor size measuring > 4 mm and upto 4mm. Our study was conducted in karachi population where the most common etiological factor was tobacco consumption and the most common site of oral cancer was buccal mucosa. So, the difference in our findings may be due to difference in anatomical site and variation in etiological factors, where as other studies the most common site was tongue(10, 11,15,17,18,19).

The strength of this study was measuring the depth of invasion for all OSCC cases by AJCC guide line. The DOI was measured for all cases, according to the current AJCC guideline, on digital H&E slides. By specialist oral pathologist. Limitation of this study was retrospective single centered study. For future recommendation A multicentered prospective

Comment [i-34]: Please rephrase this sentence. Please keep the main result (the one with the objective first)

Comment [F35]: Demographics are mentioned first so result can be better explained.

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Comment [i-38]: What is it?

Comment [F39]: Full form added

Comment [i-40]: So what is tumor thickness?

Comment [i-41]: This is more like a literature review than discussion. Needs a major modification/ revision or re-writing. The results of this study findings have to be defended in terms of language with logics and possible reasons from available literature. Not just comparison and contrast.

Comment [F42]: Modified, added

Comment [i-43]: Please mention possible source of bias as well.

Comment [F44]: Its a single centered study so it could be the possible source of bias.

study with standardized protocols should be carried out to co-relate the radiological parameters with the histological determinants with depth of invasion(18)

Table 1: age, gender and histopathological characteristics of patients

Variables Categories	Total = N (%)
Age Mean \pm SD	46.87 \pm 12.25
Gender Male Female	113 (80.7%) 27 (19.3%)
Grade of tumor Well differentiated Moderately differentiated Poorly differentiated	9 (6.4%) 125 (89.3%) 6 (4.3%)
Perineural invasion Yes No	97(69.3%) 43 (30.7%)
Lymphovascular invasion Yes No	131 (93.6%) 9 (6.4%)
Depth of invasion (up to 4mm) (>4mm)	25 (17.9%) 115 (82.1%)
T stage	
T1 T2 T3 T4	15(10.7%) 35 (25%) 20(14.3%) 70(50%)
Nodal status	
N0 N1 N2a N2b N2c N3	57 (40.7%) 25 (17.9%) 10 (7.1%) 24 (17.9%) 2 (1.4%) 21 (15%)
Tumor size	4.36 \pm 2.433
Tumor thickness	1.95 \pm 1.48
Anatomical location Buccal mucosa Tongue Others	103(73.6%) 18(12.9%) 19 (13.57%)

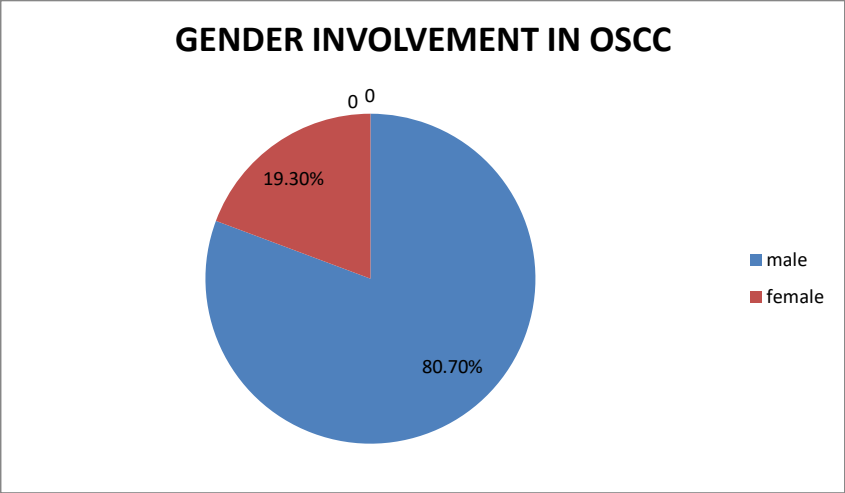


Fig. 1.Gender involvement in OSCC

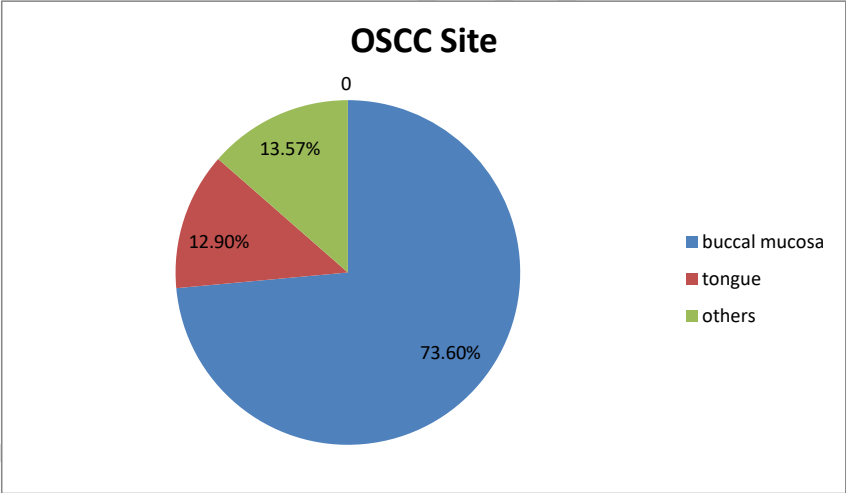


Figure 2:Frequent sites of OSCC involved

TABLE 2: Association of depth of invasion with clinico-pathological parameters

S.NO	DOI Up to 4mm	DOI >4mm	TOTAL	P value
Age Mean \pm SD 46.74 \pm 12.25	25	115	140	0.091
GENDER				0.115
MALE	23	90	113	
FEMALE	2	25	27	
T				0.001*
T1	6	9	15	
T2	1	34	35	
T3	0	20	20	
T4	18	52	70	
N				0.023*
N0	16	41	57	
N1	6	19	25	
N2a	2	8	10	
N2b	1	24	25	
N2c	0	2	2	
N3	0	21	21	
Tumor Size in mm	25	115	140	0.217
Anatomical site				0.075
Buccal mucosa	19	84	103	
Tongue	0	18	18	
Others	6	13	19	
THICKNESS IN mm	25	115	140	0.002*
GRADE				0.511
Well differentiated	3	6	9	
moderately differentiated	21	104	125	
poorly differentiated	1	5	6	
Lymphovascular invasion				0.148
Yes	0	9	9	
No	25	106	131	
Perineural invasion				0.422
YES	6	37	43	
N0	19	78	97	

4. CONCLUSION

The DOI is an important tumor predictive factor for occult neck metastasis in Oral Cancer. Our study showed significant association of DOI with lymph node metastasis. OSCC patients with a Depth of invasion >4 mm had increased the probability of lymph node metastasis than those having depth of invasion upto 4 mm.

CONSENT :

Informed written consent was obtained from patients guardian.

ETHICAL APPROVAL:

The study was accepted by the Ziauddin University Ethics Review Committee. Ethical approval was obtained (Reference Code: 0330618FAOM))It was carried out in line with the Helsinki Declaration, and all participants gave their informed written permission.

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Comment [i-[45]]: It appears like retrospective study, so how was this possible?

Comment [i-[46]]: Please make it uniform as possible.

Comment [F47]: done

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