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Correlation between Histological Depth Invasion & Metasis of Anterior 2/3rd of Tongne & Buccal Mucosa

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ABSTRACT

Background: When compared to other imaging modalities, such as CT or MRI, ultrasonography(US) offers the highest spatial resolution for soft tissues, allowing it to reveal structural detail down to the millimeter scale.

Aim: To determine and evaluate the correlation between H-DOI & CLNS of OSCC of anterior 2/3rd of tongue & BM.

Material & method: 33 patients were included for the tretament of OSCC then the probe was then placed directly on the lesion & DOI was measured in a vertical plane from the adjacent normal surface epithelium to the point of maximal depth and checked for HE.

Result: We found that , intraclass correlation was 0.981 which was highly significant depicting HE and US-DOI showed strong correlation.

Conclusion: There was a strong correlation between DOI and the occurrence of metastasis.

Keywords: DOI, HE, BM, tongue, CLNS,OSCC,US,CT, MRI.

INTRODUCTION

Early Oral Squamous Cell Carcinoma (OSCC), especially in cases when the tumors are well-differentiated and have not spread to other parts of the body, is generally considered to have the most favorable outlook. Regrettably, the majority of OSCC cases are diagnosed at an advanced stage of the disease. The five-year survival rates at an advanced stage do not exceed twelve percent of the patients. Typically, during the first thirty months of severe OSCC, the majority of patients succumb to the disease [1]. In roughly 80% of individuals, metastases from OSCC will develop in cervical lymph nodes. Usually used in these situations is a cervical lymphadenectomy (radical neck dissection). Selective neck dissection has lately been created to lower the morbidity of radical neck dissection [2]. Several variables influence the chance of cervical metastasis, including the tumor's location, size, and histological grade. Recent research suggests a relationship between the amount of invasion of OSCC and its spread to surrounding tissues [3].The use of various modalities of radiological imaging

is crucial for pre-treatment planning and determining the stage of illness in order to develop a treatment plan. Palpation-based staging of cervical lymph node metastasis has been shown to be unreliable. The incidence of hidden cervical nodal metastases is at least 30% when assessed with simple palpation [4]. The study conducted by Yesuratnam et al.[5] and Lodder et al.[6] revealed a more robust relationship between ultrasonographic total thickness (TT) and histopathologic TT when compared to measurements obtained using MRI.

AIM

To determine and assess the correlation between histological(H) depth of invasion(DOI) & cervical lymph node metastasis (CLNS) of OSCC of anterior 2/3rd of tongue & BM.

INCLUSION CRITERIA

1. Patient age group with 25-80 year.
2. Biopsy proven OSCC of BM & anterior 2/3rd of tongue.
3. Lesion whose posterior limit could be determined on clinical examination.
4. Only T1 & T2 tumor size according to AJCC classification

EXCLUSION CRITERIA

1. Patient outside 25-80 years age group.
2. Malignancy other than OSCC
3. Non-malignant lesion of oral cavity
4. Patients who were previously operated for OSCC
5. T3 , T4 tumor of AJCC classification

MATERIAL & METHOD

We have conducted prospective, comparative , clinical study in total of 33 patients in the department of Oral and Maxillofacial surgery in KIMSDU starting from January 2020 to June 2021.

Material

a. Bard Parker blade no.15
b. Scalpel
c. Adson‘s tissue holding forcep (toothed)
d. Artery forcep
e. No.3 metallic suction tip
f. Austin’s Atraumatic Retractor
g. Langenbach Retractor
h. Needle Holder
i. Suture Cutting Scissor
j. 3-0 Silk Suture

Specimens were stained using PAS (Periodic Acid-Schiff). Stain and histological depth were measured from the basement membrane of a normal adjacent level to the deepest point of invasion of the tumor in a perpendicular fashion under 40x magnification.

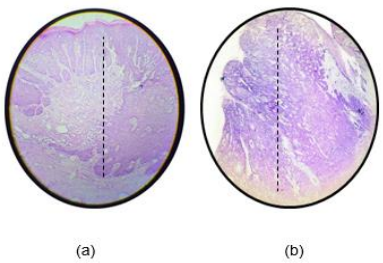


FIGURE 1: 40 X PAS STAINED HISTOLOGICAL SLIDE WITH DOI MEASUREMENT

Methodology

All the patients with suspected malignant ulcerations in oral cavity were subjected to incisional biopsy after routine blood investigation as shown in figure 2.

FIGURE. 3: PHOTOGRAPH OF SUSPECTED MALIGNANT ULCER ON RIGHT LATERAL ASPECT OF TONGUE

2% Lignocaine with Adrenaline (2.200000) was infiltrated locally around the ulcer & specimen from most respresentative site of lesion incised using 15 Blade Parker blade as shown figure 4.

FIGURE. 4: ARMAMENTARIUM FOR INCISIONAL BIOPSY

Post-operative edication & instruction given to all patients after achieving hemostasis. Furthermore, Cap. Amoxicillin 500 mg- BD , Tab. Diclofenac Sodium – BD ,Tab. Omeprazole 40 mg- OD for 5 days and Betadine Mouthwash- TID for 7 days. Incision biopsy specimen of all patients was sent for H-E. The probe was then placed directly on the lesion & DOI was measured in a vertical plane from the adjacent normal surface epithelium to the point of maximal depth as shown in figure 5 & 6.

FIGURE. 5: PHOTOGRAPH OF ULTRASONOGRAPHY BEING PERFORMED ON THE PATIENT

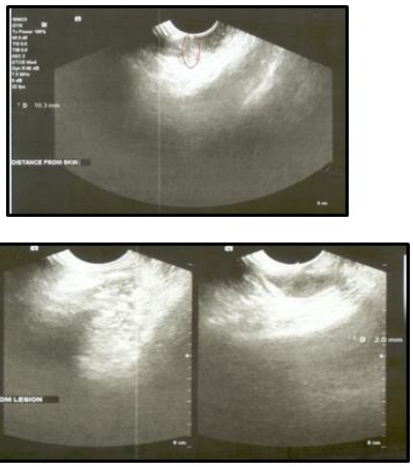
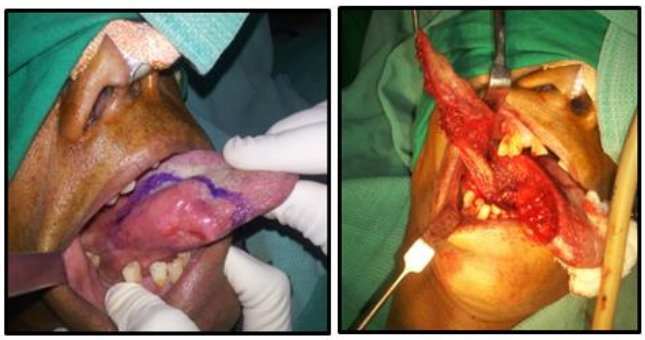


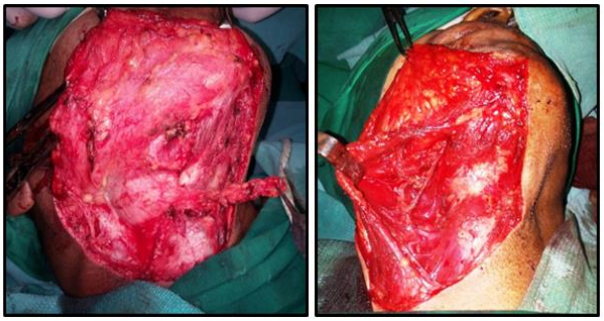
Figure. 6: Photographs of ultrasonograph of patient with measured depth of invasion

After due fitness from department of anesthesia , all patients were taken for surgery under GA.Wide excision with elective neck dissection was performed for all the patients atleast upto level III lymph nodes as shown in figure 7 & 8.



(a) (b)

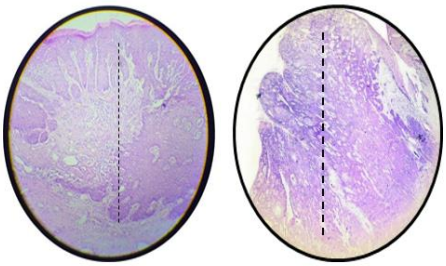
FIGURE. 7: PHOTOGRAPH OF (A) INCISION MARKING FOR WIDE EXCISION (B) WIDE EXCISION OF PRIMARY TUMOUR OF TONGUE



(a) (b)

FIGURE. 8: PHOTOGRAPH OF (A) LEVEL I AND II LYMPH NODES BEING CLEARED (B) NECK AFTER REMOVAL OF LYMPH NODES

Post- surgery , resected specimen was sent for the analysis in neutral buffered formaline to avoid tissue shrinkage. Furthermore, the specimens were stained using PAS stain & histologicla depth was measured from basement membrane of normal adjacent level to deepest point of invasion of tumour in perpendicular fashion under 40 X magnification as shown in figure 9.



(a) (b)

FIGURE 9. 40X PAS STAINED HISTOLOGICAL SLIDES WITH DOI MEASUREMENT.

The DOI of OSCC through histological reports was compared to the DOI which was obtained through preoperative US.Correlation between the depth of invasion of OSCC & cervical lymph node metastasis was investigated.

STATISTICAL ANALYSIS

Data obtained was entered in microsoft excel using SPSS . The collected data was summarized by frequency,percentage, mean & standard deviation.

RESULT

TABLE 1:MEAN AGE

	N	Minimum	Maximum	Mean	Std. Deviation
Age (in years)	33	36	78	56.27	10.849

Table 1 showed that ,the mean age of enrolled patients was 56.27 years with a standard deviation of 10.84 years. Minimum and maximum age of the patients was 36 and 78 years respectively

Age (in years)	Frequency	Percent
50 and below	11	33.3
51 - 60	8	24.2
Above 60	14	42.4
Total	33	100.0

TABLE 2: AGE DISTRIBUTION OF PATIENT

Table 2 depicted that, 14 patients (42.4%) were of age above 60 years, eight patients (24.2%) were between the age group of 51 – 60 years and eleven patients (33.3%) were of age 50 years or less.

SEX	FREQUENCY	PRECENT
Female	5	15.2
Male	28	84.8
Total	**33**	**100.0**

TABLE 3: SEX

Table 3 showed that, 5 of the 33 patients were females (15.2%), and 28 patients (84.8%) were males.

		Lymph Node Involvement			
		Yes		No	
		Count	Row N %	Count	Row N %
Age	50 and below	6	54.5%	5	45.5%
	51 – 60	6	75.0%	2	25.0%
	Above 60	9	64.3%	5	35.7%

Sex	F	3	60.0%	2	40.0%
	M	18	64.3%	10	35.7%

TABLE 4: LYMPH NODE INVOLVEMENT (LNI)

Table 4 depicted that, out of 33 patients, 21 had cervical lymph node metastasis. amongst the age group of 50 and below, 54.5% (n = 6) patients had lymph node involvement, 75% of patients (n = 6) between 51 – 60 years of age and 64.3 % of patients (n = 9) above 60 years of age had lymph node involvement. 60% (n = 3) of females and 64.3% of males (n = 18) had lymph node involvement.

Pearson Chi-Square Tests	
	p
Age	.656
Sex	.854

TABLE 5: PEARSON CHI-SQUARE TEST

Table 5 showed that , using Pearson Chi-Square test, p value for age was .656 and for sex was .854 which was statistically insignificant. Therefore, it was concluded that the demographic variables of the patient were statistically insignificant when compared with involvement of cervical lymph nodes.

	Lymph node metastasis	**N**	**Mean**	**Std. Deviation**	**t test p value**
Histological Depth of Invasion (in mm)	Yes	21	9.05	3.457	0
	No	12	4.33	0.651	

TABLE 6: MEAN HISTOLOGICAL DOI

Table 6 showed that, unpaired ‘t’ test was performed for evaluation of lymph node metastasis when compared to histological depth of invasion. The mean DOI in cases with positive lymph node metastasis (n = 21) was 9.05mm with a standard deviation of 3.45mm. In patients with negative lymph node metastasis (n = 12) mean DOI was 4.33mm with a standard deviation of .651mm. This was highly significant (p = <.001)

Clinically palpable lymph node			
		Frequency	Percent
	Absent	18	54.5
	Present	15	45.5
	Total	33	100.0

TABLE 7: NUMBER OF PATIENT WITH OR WITHOUT CLINICALLY PALPABLE LYMPH NODE.

Table 7 showed that, 54.5% of patients (n=18) patients had clinically palpable lymph node while 45.5% patients (n=15) cervical lymph nodes were not palpable.

		Frequency	Percent
	Absent	6	66.67
	Present	12	33.3
	Total	18	100.0

TABLE 8: NUMBER OF PATIENTS WITH HISTOLOGICALLY POSITIVE LYMPH NODE METASTASIS IN CLINICALLY NEGATIVE NECKS

Table 8 showed that, out of 18 patients in whom lymph nodes were not palpable clinically, 66.67% of patients (n = 12) had cervical lymph node metastasis on post operative

hisotpathological evaluation while 33.33% patients (n = 6) did not have metastatsis into lymph nodes.

Area Under the Curve				
Test Result Variable(s): Histological Depth of Invasion				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.99	0.013	0	0.964	1

TABLE 9: AREA UNDER ROC CURVE

Table 9 showed that, for obtaining a cut off value of histological DOI at which the lymph node metastasis increases significantly, Receiver operating characteristic (ROC) analysis was performed.

POSITIVE IF GREATER THAN OR EQUAL TO	SENSITIVITY	SPECIFICITY
2.00	1.000	1.000
3.50	1.000	0.917
4.50	1.000	0.417
5.50	0.952	0.000
6.50	0.667	0.000
7.50	0.524	0.000
8.50	0.476	0.000
9.50	0.381	0.000
10.50	0.286	0.000
11.50	0.238	0.000
12.50	0.190	0.000
13.50	0.143	0.000
14.50	0.095	0.000
16.00	0.048	0.000
18.00	0.000	0.000

TABLE 10: COORDINATE OF ROC CURVE

Table 10 show that, indicating true positive condition lymph node metastasis is linked with increasing histological DOI. The sensitivity at 5.5 mm of DOI ,which is 0.952, is DOI at which incidence of lymph node metastasis increases significantly. Specificity at the DOI is 100%.

	N	Mean	Std. Deviation	Mean difference	S.D of difference
Histological Depth of Invasion (in mm)	33	7.33	3.594	.515	.795
Ultrasonographic Depth of Invasion (in mm)	33	6.82	3.264		

TABLE 11: COMPARISON OF MEAN HISTOLOGICAL & UTRASONOGRAPHIC DEPTH OF INVASION

Table 11 showed that, average mean depth of invasion through ultrasonography was 6.82mm with a standard deviation of 3.26mm whereas the average mean depth of invasion through histopathology was 7.33mm with standard deviation of 3.9mm. Mean difference between utrasonographic & histopathological depth of invasion was 0.515mm with a standard deviation difference of 0.795mm.

Intraclass Correlation Coefficient			
Intraclass Correlation	95% Confidence Interval		
	Lower Bound	Upper Bound	P
.981	.937	.992	.000
	Interpretation of ICC		
<0.40	Poor Agreement		
0.4 – 0.75	Fair Agreement		
0.75 – 0.85	Good Agreement		
>0.85	Excellent Agreement		

TABLE 12: INTRA-CLASS CORRECTION COEFFICIENT FOR ULTRASONOGRAPHIC & HISTOLOGICAL DOI & ITS INTERPRETATION

Table 12 as shown that , the intraclass correlation was 0.981 which was highly significant depicting histological and ultrasonographical depth of invasions showed strong correlation.

DISCUSSION

As previously indicated, when the problem expands, it infiltrates adjacent tissue and spreads to lymph nodes in the neck region. Regarding the Tumour, Node, and metastasis staging classification, there is unanimity in the treatment strategy that advises including the neck nodes when treating larger tumors (T3 and T4) or preoperative N-positive neck nodes. The treatment for obviously evident neck metastasis is simple: surgical removal of the neck's fibroadipose and lymphatic tissues, as well as any associated structures [7,8]. Nonetheless, the greatest uncertainty in neck treatment emerges when there are no clinical symptoms of metastasis (N0). According to studies, the incidence of concealed cancer spreading to other areas of the body in oral squamous cell carcinoma (OSCC) ranges from 20% to 45% [9,10]. However, a significant number of

patients may not need such vigorous treatment, and it is related to postoperative morbidity, notably shoulder syndrome. END may also remove or eliminate an inbuilt natural barrier that prevents the progression of cancer [7,11].

Although TT and DOI are often considered to be similar, they actually have distinct differences. Thickness is typically measured by using an optical micrometer or transparent ruler placed over the slide. This measurement is taken from the mucosal surface of the tumor to the deepest point of tissue invasion, in a perpendicular manner. On the other hand, DOI (depth of invasion) is measured from the basement membrane of the adjacent normal tissue to the deepest point of tumor invasion. As the depth of invasion increases and the tumor grows, it comes closer to blood vessels and lymphatics, which enhances its ability to spread and metastasize. Unfortunately, there is a lack of preoperative availability for reliable histological factors that can be used to assess regional metastasis. Therefore, having precise knowledge of the depth of invasion before undergoing surgery would be highly beneficial.

Out of the 33 patients included in our research, 18 patients did not exhibit any clinical signs of lymph node involvement. Among the 18 patients, 12 had lymph node metastasis in the postoperative samples. In our research, the incidence of hidden spread of cancer to the lymph nodes in patients with no apparent signs of neck involvement was found to be 66.6%, which is greater than the reported rate in existing literature, which typically ranges around 40%. The primary factor contributing to this elevated prevalence is the subjective nature of clinical palpation. Palpation is a valuable assessment technique, but it presents challenges in terms of impartiality and accuracy. According to Woolgar JA, the ability of professional surgeons to accurately diagnose metastasis by clinical examination has been insufficient, with a sensitivity ranging from 60% to 70% [12]. High-quality ultrasonic images were acquired, and the tumor thickness was measured within 1 mm in research by S. Shintani et al. [13] comparing the sensitivity and specificity of USG, CT scan, and MRI. For most cancers smaller than 5.0 mm in thickness, CT and MRI did not find a substantial density difference from the normal tissue to precisely demark the area of the tumor. There was a notable link between the data collected using intraoral ultrasonic waves and the histological sections. The conducted research demonstrated that ultrasonic waves were superior to CT and MRI in assessing the primary lesion of OSCC. In a meta-analysis to evaluate USG, MRI, and CT scans in lymph node metastasis identification, R.B.J. de Bondt et al.[14] also found that USG showed better accuracy than MRI and CT scans in head and neck tumors.

The average histological depth of invasion (DI) for the 33 patients who participated in our study was 7.33 mm. The average histological depth of invasion (DI) in 21 patients with neck metastasis was 9.05 mm, whereas in 12 patients without metastasis, it was 4.33 mm. According to our research, patients with a diameter of invasion (DI) greater than 5.5 mm had a much higher risk of developing undiagnosed cervical metastasis. It was shown in 2016 by Loganathan [15] et al. that there is a substantial correlation between the prevalence of occult metastasis and a DOI >5 mm. Faisal et al. found 23% occult metastasis in patients with a DOI of 5 mm, and they recommend END even in patients with extremely early tumors [16]. In our study, demographic variables had no effect on the metastasis (the spread of cancer) of primary tumors into neck nodes.

CONCLUSION

There was a strong correlation between DOI and the occurrence of metastasis. Our study found that there is a significant increase in lymph node metastasis when the DOI reaches a cutoff value of 5.5 mm. The second purpose of our study was to assess the usefulness of pre-operative and operative ultrasonography in evaluating DOI. We concluded after thorough evaluation that USG is a highly efficient, cost-effective, and reliable technique for measuring preoperative hemodynamic index. The ultrasonographic assessment of DOI closely matched the histological DOI, which is considered the gold standard.

We recommend that for a neck with no evidence of N0 and DOI equal to or more than 5.5 mm, it is appropriate to choose elective neck dissection. We can use preoperative ultrasonography to quantify the DOI and identify N0 patients who should undergo elective neck dissection. This study assists in determining the appropriate treatment strategy for N0 necks, a longstanding therapeutic quandary that has been the subject of discussion for decades. Performing a preoperative US provides specific benefits compared to a CT scan or MRI, despite each modality having its own pros and cons. For a more precise confirmation of our results, it may be necessary to conduct a study with longer follow-up periods and a larger sample size. However, these findings may certainly serve as a foundation for future research.

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