

## Can One Predict the Incidence of Metastasis in Oral Squamous Cell Carcinoma Patients Using Tumor Thickness?

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### Authors' contributions

*This work was carried out in collaboration between all authors and all the authors read and approved the final manuscript.*

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### ABSTRACT

**Aim:** The present study was undertaken with the purpose to assess the correlation between the presence of lymph node metastases and the size of tumor and the thickness of the tumor mass.

**Methods:** The study included 80 consecutive cases of oral squamous cell carcinoma, who underwent radical neck dissection. The various level of lymph nodes in these cases were checked for metastases, which was then correlated with the size of tumor and the thickness of the tumor.

**Results:** In this study it was revealed that there is a statistically non-significant ( $p = 0.3204$ ) correlation between size of the tumor and regional cervical lymph node metastases, but a significant correlation ( $p = 0.0148$ ) between thickness of the tumor and regional cervical lymph node metastases was observed.

**Conclusion:** Thus we conclude that the tumor thickness was more in cases with skip metastasis than in cases without skip metastasis and tumors greater than 10mm in thickness have more chances of regional metastases.

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**Keywords:** Tumor thickness; tumor size; lymph node metastases; skip metastasis.

## 1. INTRODUCTION

Oral cancer is the sixth most common cancer worldwide. In India, it accounts for one fourth of male cancers and one tenth of female cancers [1]. Prognosis in common human cancers depends on tumor size and regional node metastases at the present stage of our knowledge. Distant metastases usually mean incurability, but most initial diagnoses occur before distant spread is demonstrable. The clinician faces prognostication based on the TNM system. It is yet to discover a fundamental and universal feature of tumor microscopic structure or a tumor biochemical product as reliable as tumor size and regional metastasis.

Thickness has now become the major prognosticator in melanoma, largely determining initial treatment. Depth of penetration in millimeters has been found to be a most valuable measurement in predicting tumor behavior in cancers of the uterus, especially the cervix, cancers of the gastrointestinal tract, especially the colorectum, and cancers of the bladder. Caution is appropriate in comparing thickness with surface size, because no doubt exists that, in cancer generally, very small is good and very large is bad. Tiny oral cancers with a surface diameter of around 10 mm are associated with few if any node metastases and have a very good prognosis. Conversely, very large extensive cancers involving adjacent structures can seldom be cured. Caution is needed also because depth of penetration and thickness are subtly different; depth is not always reproducible in the mouth.

The presence of micro metastasis is thought to correlate with conventional factors such as the size of the tumor and thickness of the tumor [2]. Thick tumors, which invade more deeply, have access to wider lymphatics in which tumor emboli can form more readily than in the small-caliber lymphatics of superficial areas. So tumor thickness is one of the best predictor for loco-regional metastasis and recurrence.

Aim of this study was to assess the correlation between the presence of lymph node metastases and the thickness & size of tumor.

## 2. MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology,

Sharad Pawar Dental College and Hospital, after obtaining approval from the Institutional Ethical Committee. An informed consent was obtained from all the subjects included in the study.

Eighty patients diagnosed as primary squamous cell carcinoma of the oral cavity & who underwent surgical resection along with neck dissection was included in the study.

## 3. MEASUREMENT OF TUMOR SIZE

Tumor size is an important TNM staging parameter in many solid cancers, and the largest tumor diameter has been used for many years in the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) TNM (tumor node & metastasis) staging systems. In particular, tumor staging was considered to be a predictor of overall patient's prognosis and survival [3]. The tumor size was measured with the help of automated Vernier Caliper in centimeters of the formalin fixed resected specimen (Fig. 1).



**Fig. 1. Measurement of tumor size**

## 4. MEASUREMENT OF THICKNESS OF TUMOR

Thickness concerns measuring the entire tumor mass for this an objective parameter is needed, and it can be obtained using an ocular micrometer.

Breslow A. [4], was the first to evaluate the incidence of metastasis in cutaneous melanoma which was proportional to maximal tumor thickness. Breslow defined strict criteria for measuring cutaneous melanoma (i.e. from the deepest point of invasion to the top of the granular cell layer of the overlying epidermis,

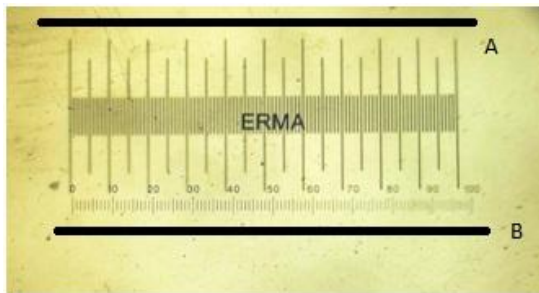
excluding keratin, parakeratin, and inflammatory exudates). If the lesion is ulcerated, the ulcer base serves as the reference point [4].

In our study gross tissue specimen were taken from the measured site (site for measurement of tumor size) of the resected specimen. The tumor thickness using the ocular micrometer was measured on the histological section, after staining with conventional H & E method.

#### 4.1 Procedure

A 10× eyepiece containing an ocular micrometer disc (Leica) and 10 × objectives were calibrated with a stage micrometer.

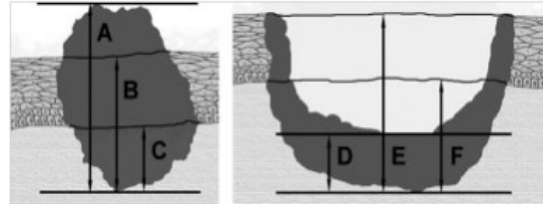
In calibration, the stage micrometer was brought into focus and moved until one of the graduations corresponded exactly with one of the divisions of the eyepiece micrometer. The true distance (A) seen on the stage micrometer, which corresponded to the number of divisions (B) of eyepiece micrometer disc, was then read, and this true distance was divided by the number of divisions subtended ( $C=A/B \times 10$ , 10 refers to the value of 1 SM division). The number of divisions covered by the section multiplied by the calibration constant (C) gave the thickness of tumor (Fig. 2).



**Fig. 2. Calibration of oculometer**

The thickness of tumor was measured from the surface of the epithelium seen under light microscope, to the point of maximum infiltration, along a straight line drawn on the slide macroscopically (An imaginary line indicating the level of the adjacent intact mucosa (Figs. 3 B–E) or of the basal membrane (Figs. 3 C–F) can be considered the starting point for measuring the thickness of the tumor into the underlying tissue to the deepest point of invasion). The thickness of tumor in this study was examined for both types of tumor i.e. exophytic and endophytic by three different examiners, independently in a

blinded fashion. It was measured along the vertical line A for exophytic tumor and vertical line D for endophytic tumor (Fig. 3).



**Fig. 3. Guidelines to measure tumor thickness**

Precautions taken: If the tumor is not cut at 90 degrees to the surface of skin/mucosa or if the processed tissue is not embedded and sectioned properly, a tangential section will be obtained with an artificial increase in the tumor thickness. [5] to minimize this error careful processing of the section was carried out. For identification of the deep infiltrating tumor cells three pathologist independently identified the infiltrating tumor cells so as to negate any bias. Care was also taken during tissue orientation during paraffin block preparation, and tissue sectioning was carried out with utmost attention to detail.

#### 4.2 Lymph Node Examination

Lymph nodes were macroscopically isolated from the patients at the time of surgery, and stained by frozen section hematoxylin and eosin method.

The remaining tissue of the lymph node was processed for paraffin- embedded sections. The paraffin- embedded lymph node was again sectioned by step serial sectioning method (50 μm sections apart) and stained for conventional H & E and Immunohistochemistry for pancytokeratin.

The following staining procedures were sequentially carried out on the sections of the lymph nodes:-

1. Intra-operative frozen sections H & E staining
2. Conventional H & E staining on formalin fixed tissue
3. Step-serial by conventional H & E staining
4. Immunohistochemical staining by Pancytokeratin antibody (Clone MNF 116, Dako, Denmark)

**5. RESULTS**

Eighty patients were enrolled during the study period. The baseline characteristics of the study participants are shown in Table 1.

**Table 1. Baseline characteristics of the study participants (N=80)**

Variable		
Mean age (SD) in years	52.3	(10.7)
Sex M:F	7:3	
Location	Frequency	Percent
Buccal mucosa	39	48.75
Floor of mouth	1	1.25
Gingivo-buccal sulcus	19	23.75
Labial mucosa	12	15
Lip & labio-gingival sulcus	2	2.5
Retromolar area	2	2.5
Tongue	5	6.25
Total	80	100.0

*Kruskal-Wallis Test (Nonparametric ANOVA); Kruskal-Wallis Statistic KW = 8.252 (corrected for ties); The P value is 0.0411, considered significant; Therefore the variation among column medians is significantly greater than expected by chance*

**5.1 Comment (Tables 2, 3)**

There is no statistically significant relation between size of tumor and occult metastasis. However there is statistically significant relation between tumor thickness and patients with occult metastasis. The thickness of tumor is more in the patients where occult metastasis is present when compared to the patients where occult metastasis is absent. It was observed that for thickness ranges between 0-5 the mean level of lymph node metastasis was 0.78, for 5.01-10.00 was 2.53/25, for 10.01-15.00 was 3.385, and for 15.01-20.00 was 4.4167. It is also observed that there is a progressive increase in lymph node metastasis with increase in the tumor thickness.

We also observe there is an increase in the regional lymph node metastasis as median thickness of the tumor increases.

**6. DISCUSSION**

Thickness and depth both emerge from the study as general indicators of tumor aggressiveness, both because of good correlation with survival and with regional node metastases and from the fact that two thirds of failures were associated with local recurrences. This would indicate greater difficulty in local eradication of thicker, more aggressive tumors. Thicker tumors may require wider margins for cure.

Literature reveals that of all the prognostic parameters that have been evaluated, tumor thickness and depth of invasion have emerged as the best predictors of ONM (Occult Nodal Metastasis) in the clinically negative neck [6].

Thickness has become the major prognosticator in melanoma, largely determining initial treatment. Depth of penetration in millimeters has been found to be most valuable measurement in predicting tumor behavior in cancers of the uterus, especially the cervix cancers of the gastrointestinal tract, especially the colorectum, and cancers of bladder [7]. Since tumor thickness has emerged as a good predictor for prognosis, we evaluated this parameter, in this study. We attempted to identify if any correlation exists between tumor thickness and lymph node metastasis (occult/obvious) in cases with Oral Squamous cell carcinoma.

The term “skip metastasis” refers to nodes involved with cancer which are not in the orderly progression of the disease, or what we refer to as “the first echelon of nodes.” (Byers et al. 1997). In a normal lymphatic process the tumor emboli are disseminated in the lymphatic system in an orderly fashion, beginning with the sentinel

**Table 2. Correlation between size of tumor & level of lymph node involvement by Pearson’s linear correlation**

Pearson correlation coefficient (r)	95% confidence interval	p-value	Significance
-0.1125	-0.3243 to 0.1100	0.3204	Not significant

**Table 3. Correlation between thickness of tumor & level of lymph node involvement by Pearson’s linear correlation**

Correlation coefficient (r)	95% confidence interval	p-value	Significance or not
0.2716	0.05515 to 0.4637	0.0148	Significant

lymph node(s) [SLN] or the primary lymph node and then to the remaining lymph nodes within the nodal basin. In skip metastases the tumor emboli disseminate to secondary node rather than the primary or the first echelon of nodes.

Tumor thickness is measured as the distance from the surface of the tumor to the maximum depth of infiltration. A distinction needs to be made at the outset between the depth of penetration of a cancer and thickness. Depth of penetration means extent of cancer growth into tissues beneath an epithelial surface, where epithelium is destroyed (some investigators reconstruct a surface line and measure from this) [8]. Penetration sometimes is expressed with reference to microscopic anatomic structures reached, rather than by micrometer measurements in millimeters. Thickness, on the other hand, is a direct micrometer measurement by the pathologist of the vertical bulk of tumor regardless of the histologic structure of the ulcerative or exophytic form of tumor growth [9].

In this study it was revealed that there is a statistically significant correlation between thickness of the tumor and regional cervical lymph node metastases. It was observed that for thickness ranges between 0-5 the mean level of lymph node metastasis was 0.78, for 5.01-10.00 was 2.53/25, for 10.01-15.00 was 3.385, and for 15.01-20.00 was 4.4167. It is indicating progressive increase in (Tables 4, 5) lymph node metastasis with increase in the tumor thickness. Thus we can take 10 mm thickness as Cut-off value of tumor thickness for predicting metastasis.

**Table 4. Showing correlation between level of Lymph node Involvement at different tumor thickness**

Thickness of tumor= d	Mean lymph node involvement
0-5	0.78
5.01-10.00	2.53125
10.01-15.00	3.385
15.01-20.00	4.4167
20.01-25.00	1

Our study results are in agreement to that of Yunen et al, who suggested that among all the tumor parameters and predictive models being evaluated, tumor thickness was the only significant factor that had significant predictive

value for subclinical nodal metastasis [10]. Lim et al., Hayashi T et al., Onerci M et al., Asakage T et al. and many others also suggested that tumor thickness had a predictive value for cervical metastasis [11-14]. There are no similar studies on patients with OSCC which evaluated a correlation between tumor thickness and cervical metastases. But then in various studies the tumor thickness cut off value differed which may be attributable to many factors, such as the definition of tumor thickness and oblique section in processing surgical specimen [11]. The first step in the measurement of tumor thickness is identification of the most deeply infiltrating tumor cells, which is a subjective phenomenon. It could explain the variation in tumor thickness, depending on individual's view.

The unique feature of our study is that we made an attempt to correlate thickness of tumor with mean lymph node involvement. This comparison revealed a positive correlation between thickness with mean lymph node involvement. We also observed statistically significant difference between tumor thickness of patient with skip metastasis and without skip metastasis i.e. the tumor thickness was more in cases with skip metastasis than in cases without skip metastasis.

The probable reason for increase metastases, could be that the thick tumors, which invade more deeply, have access to wider lymphatics in which tumor emboli can form more readily than in the small-caliber lymphatics of superficial areas [15]. This finding is in agreement with Anthony Sparano et al. [16] and many authors who have suggested that tumor thickness can serve as a predictive factor for increased probability of occult neck disease. The study is not in agreement with studies done by Amaral et al. and Goer kem et al. where tumor thickness failed to achieve statistical significance for prediction of occult metastasis.

We have also assessed if there is any correlation between tumor size and lymph node metastases. We took into consideration the largest tumor diameter as size of tumor. This is because solid cancer is a three dimensional structure in which cancer cell spread in different planes at various rates to invade the surrounding structures [3]. There was no statistically significant correlation observed between tumor size and cervical lymph node metastasis nor tumor size and skip metastasis.

**Table 5. Showing correlation between regional lymph node metastases and median tumor thickness**

Group	Number of points	Median	Minimum	Maximum
0-5	9	1.000	0.000	2.000
5.01-10.00	32	1.000	0.000	9.000
10.01-15.00	26	2.000	0.000	9.000
15.01-20.00	12	4.500	0.000	9.000

*The table shows there is an increase in the regional lymph node metastasis as median thickness of the tumor increases*

This may be explained by the fact tumor grows at unequal rate in different planes and in the presence of tumor necrosis, tumor never conform to a perfect spherical shape. The largest tumor diameter therefore cannot reflect perfectly the total tumor volume and total number of cancer cells [17].

The study results are in agreement with authors like Mehmet Haksever et al., Y. W. Chen et al. However Carter et al found a linear relation between tumor diameter and the percent of cases with positive lymph node involvement. Tumor size only assesses the extent of the tumor in the greatest surface dimension but overlooks the invasiveness of the tumor in the vertical plane [18]. So this might explain the absence of correlation between tumor size and metastases.

**7. CONCLUSION**

Thus the study revealed that, tumor thickness is statistically significant prognostic character which shows correlation with metastases and we can take 10 mm thickness as Cut-off value of tumor thickness for predicting skip metastasis. So one can predict incidence of metastases using thickness as a tool.

**8. FURTHER SCOPE**

Metastatic potential of oral squamous cell carcinoma varies from site-to-site. Patient may be divided into groups on the basis of location within the oral cavity.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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