Original Article

Mitosis – predicting primary tumour and metastatic lymph node behaviour and beyond in OSCC – A pilot study

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Abstract

Background and Objectives: In the evaluation of oral cancer, mitotic index/mitotic figures are of paramount importance in histopathology. The number of mitoses in tumour cells is directly proportional to the activity of the tumour cells. In this novel study, we evaluated the status of the mitotic figures found in the metastatic deposit of the lymph nodes in addition to the primary tumour. A great deal of significance is given to this parameter in the evaluation of progress of the primary tumours in a state of distant metastasis. Therefore, we propose to analyse the lymph node mitotic index (LMI) in metastatic deposits and its relation to the primary tumour by assessing the relation between primary: LN mitosis ratio (PLMR). This also can provide an insight into the concept of understanding the dynamic state of lymph node deposits in oral squamous cell carcinoma (OSCC).

Materials and Methods: A total of 510 lymph nodes from 17 cases of surgically treated OSCC were analysed, of which 40 lymph nodes were found to be positive. These 40 lymph nodes were examined histopathologically for the presence of mitotic figures in ten randomly selected fields (x400) and compared to the mitotic activity of the primary tumour by two observers independently, along with other histopathological features. LMI and PLMR were further determined for these cases.

Observations and Results: The mitotic index was significantly higher in the metastatic deposits than in the primary tumour ($P = 0.004$). A higher percentage of abnormal mitoses was observed in metastatic lymph nodes, but no statistical significance ($P = 0.27$) was found when compared with primary tumour. Statistically significant correlation ($P = 0.001$) was observed between abnormal mitoses (%) and presence of ECS as well as size of the metastatic deposits. The LMI ratio showed a high mitotic rate in close to 80% of the cases. PLMR showed 58–60% cases with mitotically more active lymph nodes.

Interpretation and Conclusion: This study is the first of its kind, which examines a level further than the nature of the primary tumour. Mitotic index/numbers in lymph nodes (LMI) might be considered an important parameter to evaluate the disease spread, revealing the nature of transiently indolent and mitotically active metastatic lymph nodes. The PLMR adds a further dimension on the lymph node's dynamic status in the progression of the disease.

Keywords: Abnormal mitosis, increased mitosis, lymph node mitotic index (LMI), metastatic lymph nodes, modified Anneroth's grading system, oral squamous cell carcinoma, primary and lymph node mitosis ratio (PLMR)

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) remains a tough malignancy to predict and tackle even in the 21st century. This malignancy still holds a precarious 50% morbidity and mortality rate[1,2] and close to 20–40% cases with occult metastasis. Lymph node metastasis has been established as the most highly adverse independent prognostic factor in OSCC.[3-5]

The TNM staging stands tall, for around 70 years in guiding the treatment protocol, but large vacuum still exists in the histopathologic findings of OSCC. Modified Anneroth's grading system and invasive edge grading systems are a few newer compilations associated with histopathology.[6]

Mitosis in the tumour cells speak of a rapid proliferation rate and cellular turnover and the presence of apoptosis is depictive of elimination of cells with damaged DNA as a protective process.[7,8] A discrepancy between proliferation and apoptosis is on the tenets for carcinomas.[8] Mitosis independently is a valuable parameter used for diagnosis and prognosis.[9]

Mitotic index is an integral segment of the histopathological grading systems routinely employed in OSCC reporting.[10] Several studies have assessed the various grading systems for prediction of lymph node metastasis.[10-12]

Analysing the mitotic rate, the presence of normal or abnormal mitoses in OSCC is of diagnostic and prognostic significance in the detection and propensity to spread further and its prediction. Numerous studies have analysed the mitotic rate and its value in prognostication of oral cancer.[13,14]

Detecting the presence of high mitotic rate or mitosis in the metastatic deposits of the lymph node also spells out a higher propensity to further metastasis and spread of the disease.

Hence, the aim of this study was to examine and record mitosis (number and type) in primary tumour mass and also in lymph node metastatic deposits. The aim was also to correlate the presence of this parameter with the presence of metastasis in the lymphatic chains of the head and neck, pertaining to its levels, correlate with other histologic features, and develop an index based on our findings.

MATERIALS AND METHODS

A retrospective study was conducted on cases obtained from the archives of Care Oral Pathology Services, Bangalore, from 2020 to 2024 on the H&E-stained sections of treated cases of OSCCs with RND, which were graded with modified Anneroth's grading system and staged using pTNM classification.

Seventeen cases of OSCC treated by radical neck dissection or modified supra-omohyoid neck dissection (RND or SOND) were selected. A total of 510 harvested lymph nodes were analysed, in which n = 40 lymph nodes were positive for metastatic deposits. These 40 positive lymph nodes were evaluated for the presence of mitosis.

Evaluation of mitoses was carried out by two observers (SM and RMB) independently. The agreement between both observers carried a strong concordance (kappa = 0.79) and was statistically significant at $P = 0.001$.

An observation of mitotic figures was based on the primary absence of the nuclear membrane and spread and extension of nuclear material in the cell body or cytoplasm along with identifiable stages of mitosis.

The numbers of mitotic figures were counted in the area of invasive edge and active fronts of the primary tumour.

In the lymph nodes, randomly selected areas of the OSCC deposits were used – ten fields under high power (x400 magnification) were considered for observing and identifying the number of mitoses.

A mitotic score of <10, 10–20, and >20 mitoses/10HPF was assigned to each of the slides assessed and compared with that of the primary tumour. The lymph node mitotic activity (LMI) was assigned as Grade I, Grade II, and Grade III, respectively. The primary and lymph node mitosis ratio (PLMR) was set up with a concept of mitotic rate in the primary tumour: mitotic rate in the metastatic lymph node. It is assigned as Type 1 (P > LN), Type 2 (P = LN), and Type 3 (P < LN).

Statistical analysis

Descriptive analysis of all the explanatory and outcome parameters was done using frequency and proportions for categorical variables and using mean and SD for continuous variables.

Chi-square test was employed to compare mitotic rates between primary and LN deposits and normal and abnormal mitosis levels between primary tumours and metastatic LN deposit and correlate these parameters with histopathological grade, extracapsular spread, and type of deposits in the LN metastasis.

The level of significance was set at $P < 0.05$.

The SPSS statistical software package (Version 22.0 IBM Corp., Armonk, NY) was used to perform statistical analyses.

OBSERVATIONS AND RESULTS

Out of the 17 cases, 10 (55.8%) were male and 7 (41.2%) were female. The mean age of the patients was 55.82 ± 12.61 years. 29.4% of the cases were from the tongue, and 17.6% from buccal mucosa, gingivo buccal sulcus, and retromolar area, respectively, 11.8% from the alveolus, and 5.9% from the lingual vestibule.

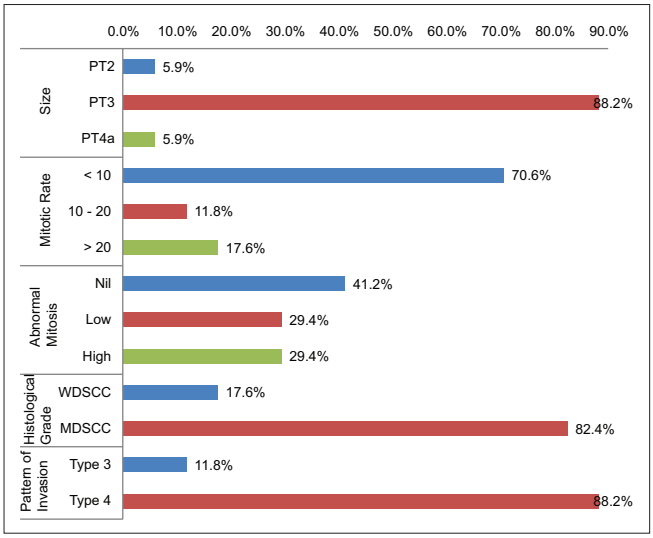
Among the 17 cases of OSCC evaluated, 510 lymph nodes were screened for metastatic deposits. The presence of metastasis was detected in 40 lymph nodes, that is, in 7.8% of the total harvested lymph nodes.

Interestingly, in 88.2% of the cases (15 of 17), the tumour size was pT3; in one case each (5.9%), the tumour size was greater, that is, pT4a; and in one case, the size was pT2 [Graph 1].

The primary tumours with lymph node metastases were majorly moderately differentiated SCC (MDSCC) in 14 of 17 cases (82.4%) and well differentiated (WDSCC) in only 3 of 17 cases (17.6%) [Graph 1].

Of the 17 cases evaluated, the rate of positive lymph nodes per case ranged from 3 to 22% of the total removed lymph nodes. These cases showed the presence of metastatic deposits in 40 lymph nodes present at L I (47.5%), L II (35%), L III (10%), and LIV (7.5%) cases, respectively [Table 1].

Type 4 invasive edge pattern (modified Anneroth's grading system) was the predominant pattern of invasion observed in 82.4% of the cases (14 of 17), and only 17.6% cases (3 of 17) showed type 3 invasive pattern [Graph 1].



Graph 1: Distribution of Histopathological characteristics of primary tumour among study samples

Among the three cases of WDSCC, the metastatic deposit also showed well-differentiated areas, whereas in the other 14 cases, five cases of MDSCC at the primary site showed well-differentiated areas in the lymph node metastasis and nine showed moderate differentiation in the deposit similar to the primary tumour [Graph 1].

Among the 40 LNs, 29 cases showed the presence of ECS in the metastatic deposits (72.5%). The nature of the deposits was macro in 25 LNs (62.5%) and micro in 15 LNs (37.5%) [Table 1].

To evaluate the activity and behaviour of the tumour cells in the lymph node metastasis, the assessment of mitosis in the tumour cells of the deposits was carried out.

Antecedently, the mitotic activity and the invasive edge pattern of the primary tumour in all the cases were determined. It was observed that in the 17 cases, 12 cases showed <10 mitoses (70.5%) – Grade 1 LMI, 2 cases – 10 to 20 mitoses (11.7%) – Grade 2 LMI and 3 cases (17.6%) – Grade 3 LMI showed more than 20 mitoses per 10 HPF (high power fields) [Graph 1].

A detailed analysis showed that the lymph node metastases showed 20% (8 nodes) with <10 mitotic figures/10 HPF (Grade I LMI), 35% (14 nodes) with 10–20 mitotic figures/10HPF (Grade II LMI), and 45% (18 nodes) with >20 mitotic figures/10HPF (Grade III LMI) [Figure 1].

Upon comparison of the mitotic rate between the primary tumour and the lymph node deposits, a statistically significant correlation was obtained [Table 2].

This indicates that there was a statistically significant difference in mitotic rates between primary tumours and metastatic lymph node deposits at $P = 0.004$ and suggests that the lymph node deposits showed a higher rate of mitosis when compared to the primary tumours.

Table 1: Distribution of histopathological characteristics of lymph node metastasis among study samples

Variable	Category	*n*	%
LN level	L I	19	47.5%
	L II	14	35.0%
	L III	4	10.0%
	L IV	3	7.5%
Extra-capsular spread	Yes	25	62.5%
	No	15	37.5%
Deposits	Micro	17	42.5%
	Macro	23	57.5%

In 23.5% of the cases, the mitotic activity of the primary tumour and the lymph node was similar (Type 2 PLMR). In approximately 58.8% of cases, the mitotic activity was higher than that of the primary tumour (Type 3 PLMR). Only 17.6% of the cases showed a reduction or falling number of mitotic figures in the lymph node metastasis as compared to the primary tumour (Type 1 PLMR) [Figure 2].

Table 2: Comparison of mitotic rate between primary tumours and metastatic LN deposit using Chi-square test

Variable	Category	Pri. Tumour - <10		Pri. Tumour - 10-20		Pri. Tumour - >20		*P*
		n	%	*n*	%	*n*	%	
Lymph Node Mitotic Rate	<10	4	14.8%	4	80.0%	0	0.0%	0.004*
	10-20	11	40.7%	1	20.0%	2	25.0%	
	>20	12	44.4%	0	0.0%	6	75.0%	

*Statistically Significant

The mitotic rate of the secondary deposits was compared to the levels of lymph nodes, but we found the correlation to be statistically insignificant ($P = 0.82$).

Apart from mitotic rate, the percentage of normal and abnormal mitotic figures was also assessed between the primary tumour and lymph node deposits.

We observed that there are evident variations in mitotic activity in the form of normal and abnormal mitoses between primary tumours and lymph node

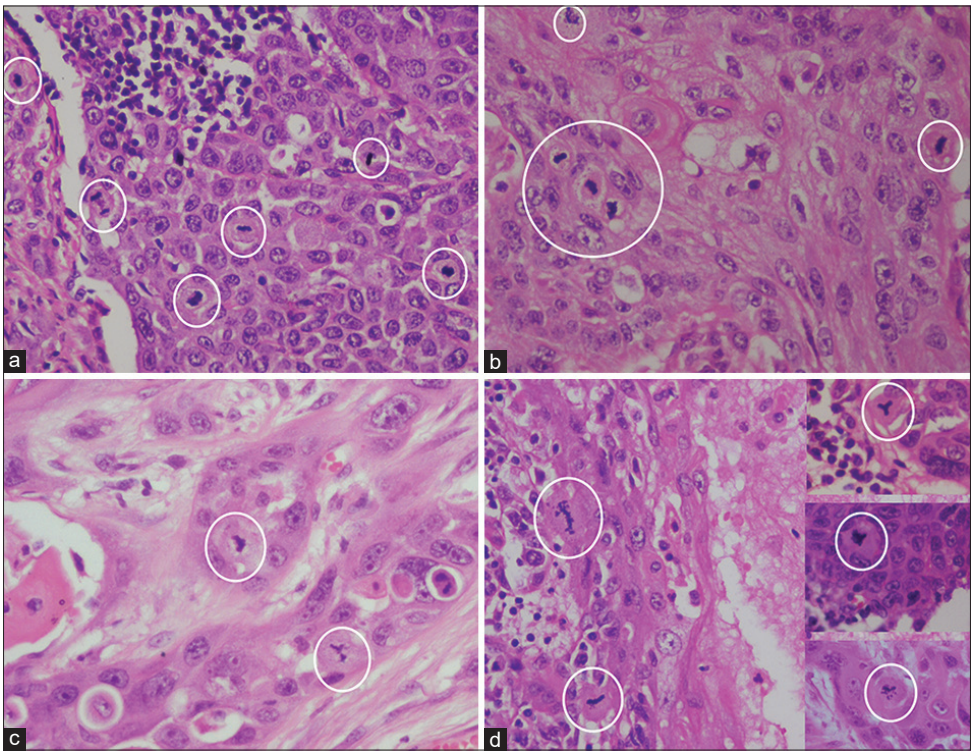


Figure 1: Presence of multiple normal (a x40, b x100) and abnormal (c x100, d x200) mitotic figures in the lymph node deposits

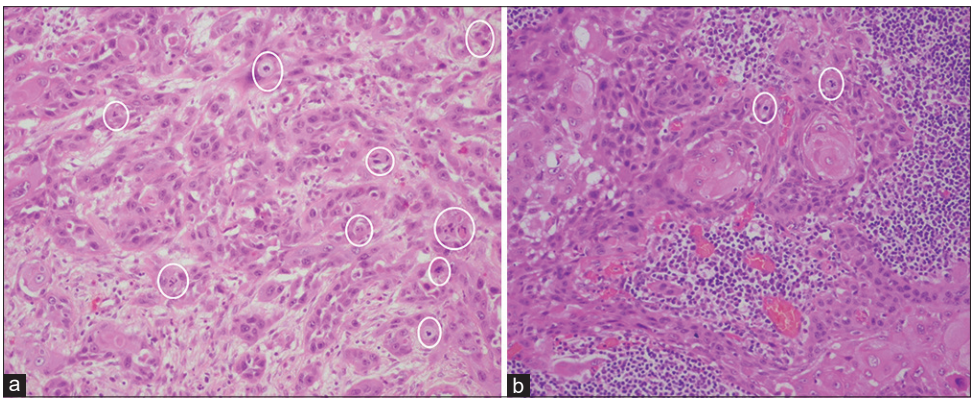


Figure 2: Metastatic deposits in lymph node exhibiting mitotically active foci (a) x100; Indolent foci (b) x100

Table 3: Comparison of Extracapsular Spread and Type of Deposits based on the Mitosis in Lymph nodes using Chi-Square Test

Variable	Category	Nil		Low		Moderate		High		*P*
		n	%	*n*	%	*n*	%	*n*	%	
Extra-Capsular Spread	Yes	1	12.5%	3	50.0%	3	50.0%	18	90.0%	0.001*
	No	7	87.5%	3	50.0%	3	50.0%	2	10.0%	
Deposits in LN	Micro	7	87.5%	5	83.3%	2	33.3%	3	15.0%	0.001*
	Macro	1	12.5%	1	16.7%	4	66.7%	17	85.0%	

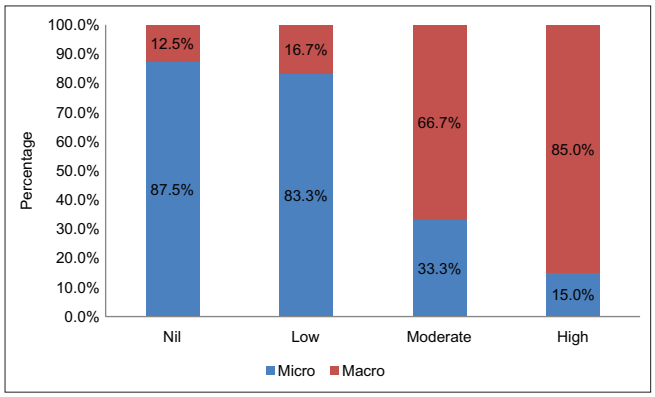
*Statistically Significant

Table 4: Lymph node Mitotic Index (LMI)

Mitotic index	Grade	Present study
<10	Grade I	20%
10-20	Grade II	35%
> 20	Grade III	45%

Table 5: Primary: Lymph node Mitosis Ratio (PLMR)

Mitotic rate	Ratio	Present study
P > LN	Type 1	17.6%
P = LN	Type 2	23.5%
P < LN	Type 3	58.8%



Graph 2: Type of deposits based on the abnormal mitosis in lymph nodes

deposits, but these differences were not statistically significant ($P = 0.27$).

When the size of the deposits was taken into consideration, normal mitoses were found more commonly in the micro-deposits and a greater percentage of abnormal mitoses was found in macro-deposits and in areas where the secondary deposit had almost effaced the whole lymph node [Graph 2].

At lymph node levels II and III, 57–75% exhibited high levels of abnormal mitosis. This indicates a very high presence of abnormal mitosis, in the deposits, though statistical significance was not achieved across all the levels ($P = 0.25$).

When the type of mitoses and ECS of the LN was correlated, when normal mitotic figures were observed in the LNs, only 12.5% showed ECS, whereas in the case of increase in the presence of abnormal mitosis, >50–90% of the lymph nodes revealed the presence of ECS [Table 3].

A statistically significant association between the presence of increased numbers of abnormal mitoses and extracapsular spread was found ($P = 0.001$) [Graph 3].

We also observed that maximum lymph nodes (87.5%) with micro-metastasis showed normal mitotic figures, whereas 85% of the positive nodes with predominantly high abnormal mitoses exhibited macro-metastasis, which was highly statistically significant ($P = 0.001$) [Table 3].

Based on all the histologic and mitotic parameters studied, we propose two indices.

The LMI ratio was seen to be high (Grade 2 and Grade 3), depicting metastatically active lymph nodes at the cellular level [Table 4].

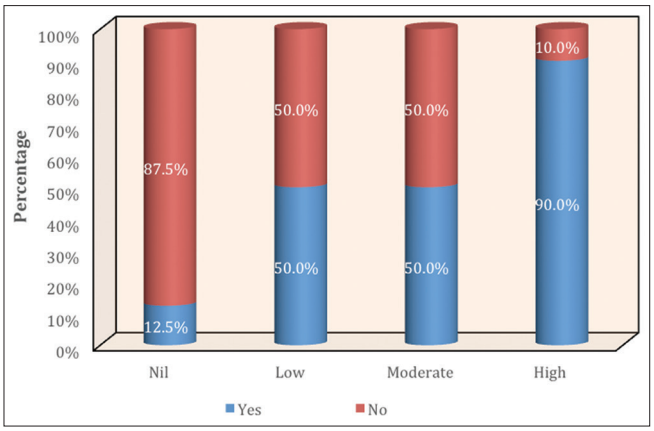
PLMR was predominantly Type 3 accounting for close to 58–60%, showing the metastatic deposit to be more active than the primary tumour. This parameter certainly needs to be addressed to curb the spread of the disease and to improve the prognosis, translation of which might change the morbidity and mortality rates [Table 5].

DISCUSSION

OSCC is the seventh most prevalent cancer worldwide, according to GLOBOCAN 2020.[15] This disease is associated with a precarious morbidity and mortality rate of 44–60%. Clinical TNM staging remains the mainstay of treatment planning for OSCC cases and to estimate the treatment response in patients.[11,15]

OSCC is treated mainly by surgery in stages I and II and by surgery in addition with adjunctive therapy in stage III and stage IV tumour category. Despite the several treatment options available, the overall 5-year survival rate after treatment of OSCC is approximately 50–52%.[1,16]

Loco-regional spread and recurrence are the most common reasons for failure of treatment. Recurrence was found to occur in about 35% of patients treated for OSCC.[16,17]



Graph 3: Extra-capsular spread based on the abnormal mitosis in lymph nodes

Although there have been several advancements in understanding molecular pathways of OSCC and instituting targetoid drug therapies, the prognosis of patients with lymph node metastasis (LNM) has remained poor.

Tumor size and extent, nodal involvement, and presence or absence of loco-regional metastasis hold a vital role in the prognosis of OSCC patients.[18] This finding is in agreement with other several studies that reported the poor prognosis of oral cancer patients who presented with T3 or T4 stage.[16]

Similarly in the present study, in 88.2% of the primary tumour cases (15 of 17), the tumour size was pT3, in one case each (5.9%), the tumour size was greater, that is, pT4a, and in one case, the size was pT2. Barboza C *et al.* and Alvi A *et al.*[18,19] in their studies highlighted that T3 and T4 tumours showed poor prognosis.

Apart from tumour size, the existence of loco-regional lymph node metastasis is deemed as the single most important prognosticator for patient outcome.[20]

In recent studies, evidence suggests that a relation between histologic grade and the development of distant metastasis (DM) in head and neck SCC exists. Fortin *et al.*[21] found that patients with grade G3 and N1 to N3 had a higher risk of DM. Garavello *et al.*[22] advocated that histologic grade was an independent risk factor for DM in HNSCC. Lim *et al.* found that patients with clinically positive neck nodes and grade G3 were more likely to develop DM in a subset analysis of head and neck SCC.

The primary tumours with lymph node metastases were majorly MDSCC in 14 of 17 cases (82.4%) and WDSCC in only 3 of 17 cases (17.6%). This fact was corroborated in studies by Fortin *et al.,* Garavello *et al.*, Doshi P, and Sumioka S *et al.*[6,21-23]

Among the three cases of WDSCC, the metastatic deposit also showed well-differentiated areas, whereas in the other 14 cases, five cases of MDSCC at the primary site showed well-differentiated areas in the lymph node metastasis and nine showed moderate differentiation in the deposit similar to the primary tumour.

These findings stress upon the poorer differentiation found in the metastatic deposits as compared to the primary tumour.

Consistently, most of the cases exhibited Type 4 invasive edge pattern (modified Anneroth's grading system) in 82.4% of the cases 14 of 17, and 17.6% cases 3 of 17 showed type 3 invasive pattern, garnering further proof that Type IV invasive pattern has a propensity to develop metastasis, confirmed by Mishra *et al.*[24,25]

The 17 cases showing lymph node metastases were categorised as WDSCC in 3 of 17 cases (17.6%) and MDSCC in 14 of 17 cases (82.4%). These cases showed the presence of metastatic deposits in 40 lymph nodes present at L I (19 cases, 47.5%), L II (35%), L III (10%), and LIV (7.5%) cases, respectively, which could predict the possibility of further spread or recurrence of the tumour.

Among the 40 LNs, 29 cases showed the presence of ECS in the metastatic deposits (72.5%). The nature of the deposits was macro in 25 LNs (62.5%) and micro in 15 LNs (37.5%). Shaw JP *et al.*[26] in their study concluded that the presence of ECS not only doubles local recurrence and distant metastasis but also triples the risk of regional failure.

All grading systems use mitoses as a pertinent feature. Unfortunately, in head and neck cancers, a clear relation between histologic grade and clinical prognosis is yet to be established, though poorly differentiated and undifferentiated SCCs have a predominance of immature cells, associated with numerous typical and atypical mitoses associated with growth and spread.[23]

In the present study, in agreement of the previous statement made, metastatic deposits to the lymph node were assessed for mitotic activity. A total of 510 lymph nodes were screened for metastasis, from the 17 cases of OSCC evaluated. The presence of metastatic deposits was detected in 40 lymph nodes, that is, in 7.8% of the total harvested lymph nodes.

Counting of mitotic figures is the oldest, valid, quick, practical, and economical way of assessing proliferation on an H&E slide. An increased number and abnormal mitosis indicate genetic damage. Thus, identification and quantitation of mitotic cells form an invaluable part of histological grading systems, which contributes to the assessment of prognosis. They can be identified and counted under a conventional light microscope.[27,28]

Defects of mitosis result in various nuclear abnormalities, namely, micronuclei, binucleation, broken egg appearance, pyknotic nuclei, and increased numbers of and/or abnormal mitotic figures.[29,30]

These abnormal mitotic figures (MFs) are commonly seen in oral epithelial dysplasia and SCC. Location and increased numbers of and/or abnormal mitotic figures are important criteria that carry increased weightage in the grading of dysplasias.[29]

Though Bryne *et al*.[31] initially included mitotic count as one of the parameters; later, some authors omitted mitotic count parameter, but still, close correlation between pattern of invasion and metastasis as well as recurrence were shown. Though some studies pointed out the complication of standardising the mitotic count in any recording system, *Sharma P et al*. and *Dissanayake et al*.[2,32] did not report any difficulty in standardising the same.

Counting of mitotic figures is deemed valuable for diagnosis and prognostication of various malignancies including breast, gastric, and neuroendocrine carcinomas.[33]

Vila CS *et al*. in a similar study on OSCC concluded that a mitotic index of <10/10HPF correlated with a well-differentiated tumour, whereas poorly differentiated tumours showed an index of >20/10 HPF and moderately differentiated tumours had an index between 10 and 20.[34]

When the mitotic activity and the invasive edge pattern of the primary tumour in all the cases were determined, it was observed that in the 17 cases, 12 cases showed <10 mitoses (70.6%), Grade I LMI; 2 cases showed 10 to 20 mitoses (11.8%), Grade II LMI; and 3 cases (17.6%) showed >20 mitoses per 10 HPF, Grade III LMI.

A detailed analysis of the current study showed that the lymph node metastases showed 20% (8 nodes) with <10 mitotic figures/10 HPF, Grade I LMI; 35% (14 nodes) with 10–20 mitotic figures/10HPF, Grade II LMI; and 45% (18 nodes) with >20 mitotic figures/10HPF, Grade III LMI.

This index, when compared with the primary tumour and the lymph node deposits, was found to be definitely higher in the secondary deposits and was found to be statistically significant in the present study ($P = 0.004$), suggesting a positive correlation of higher proliferative activity in the lymph node deposits.

This effectively states that around 75–80% of the metastatic deposits are in an active state of tumour cell multiplication, potent enough to propagate the disease further. The other 20–25% of the deposits are in a passive state, which can sequel in into a state of indolence or may change into an active stage at a later date.

When mitotic index was correlated with the level of lymph nodes, though the X^2 test was insignificant ($P = 0.82$), we found higher rates in level II and III, highlighting a higher prevalence of active mitosis.

Nevertheless, it becomes very clear that active lymph nodes have a propensity for further spread. This histopathologic detailing necessitates a thorough analysis of prognosis and treatment protocol for each patient.

In 23.5% of the cases, the mitotic activity of the primary tumour and the lymph node was similar (Type 2 PLMR).

In approximately 58.8% of cases, the mitotic activity was higher than that of the primary tumour (Type 3 PLMR). Only 17.6% of the cases showed a reduction or falling number of mitotic figures in the lymph node metastasis as compared to the primary tumour (Type 1 PLMR).

This ratio between the primary and lymph node mitotic activity can be notated as the PLMR (primary: lymph node mitosis ratio), which can be used an indicator of the activity of the metastatic deposit.

This may suggest that the tumour cells in the lymph node have a higher propensity for further spread and establishing newer niches in other loco-regional or distant sites.

Several authors have correlated the presence of high mitotic activity and proliferation rate with the prognosis of the tumour and lymph node metastasis.[14,35-37] Kapoor *et al*.[38] in their study stated that mitotic figures can be abnormally high in OSCC as mitotic index is a measure of the proliferative activity of tissue.

Similar findings were seen in the present study, and the same parameter was proposed for further analysis of the metastatic lymph node, which showed >45% of the positive

nodes showed a lymph node mitotic index of >20 (Grade III LMI), predicting a poorer prognosis and higher chance of recurrence and further spread in such cases.

A thorough literature search revealed that so far, no other studies have assessed the importance of LMI in predicting recurrence and DM in OSCC.

Mitotic figures can be either normal or abnormal. Abnormal or atypical mitosis refers to the presence of unusual, dysregulated, and random assembly of nuclear materials within the dividing cells, which results in uncharacteristic mitotic morphology which also reflects underlying genomic abnormalities such as chromosomal instability, telomere dysfunction, and aneuploidy.[29,39,40]

In the present study, the occurrence of normal and abnormal mitotic figures was also assessed between the primary tumour and lymph node deposits.

A higher percentage of abnormal mitoses could be observed in the metastatic deposits when compared to the primary tumour, indicating that the OSCC cells in the lymph nodes are definitely more aggressive and harbour more chromosomal defects, depicting dysplastic cells undergoing anaplastic change.

In our study, this correlation did not give us a statistical significance ($P = 0.27$), suggesting that there is a wide variation in the type of mitosis occurring in the primary tumour and lymph node deposits.

But a statistically significant correlation could be observed in terms of ECS and size of metastatic deposit wherein the percentage of ECS in the lymph nodes was low in those with normal mitotic figures and a higher percentage of ECS was found in the lymph nodes exhibiting a high amount of abnormal mitoses ($P = 0.001$), suggesting that higher mitosis in lymph nodes is strongly associated with the presence of extracapsular spread.

Similarly, maximum lymph nodes (87.5%) with micrometastasis showed more normal mitotic figures, whereas 85% of the positive nodes with predominantly high abnormal mitoses exhibited macro-metastasis, which was highly statistically significant. This might indicate that a higher ratio of abnormal mitosis in lymph nodes is strongly associated with the presence of macro deposits ($P = 0.001$).

These findings highlight the significance of mitotic activity in predicting both extracapsular spread and the type of metastatic deposits in lymph nodes.

Another interesting finding in our study showed higher rates of abnormal mitoses in Level III lymph nodes when compared to Level I and Level II and in MDSCC higher prevalence of elevated abnormal mitotic activity in lymph nodes associated with MDSCC.

Atypical mitoses are thus an indicator of poor outcome and may also be a potential predictor of recurrence and further spread of the disease.[40,41]

This may possibly lead to the use of anti-mitotic drugs to reduce the proliferation rate of these mitoses and to check the spread of the tumour in general not only in the primary tumour but also in the metastatic deposits in lymph nodes and distant sites.

Loco-regional metastasis occurs by following the PUMP principle (preparation, unleash, migration, and planting) as described by Cao *et al*.[42]

Our study is the first of its kind, which attempts to discover the implications of "post-planting" by assessing the mitotic activity, which could be indicative of further proliferation of the deposit and its propagation.

It is vital to understand the dynamics, of proliferation of tumour, in the lymph nodes as they might become the future sights of monitoring the disease for better restraint of the tumour spread and hence the prognosis.

Assessment of LMI could give us a prediction value in the last rung of the PRECISE model (prevention, radiology, pre-operative evaluation, chemotherapy, immunotherapy, surgery, and post-operative evaluation), discussed by Cao *et al*.[42] for post-treatment evaluation and augmentation of adjuvant treatment.

The limitations of this study can be overcome by recruiting a higher number of cases. Better correlation and reproducibility of this study can be achieved by standardising 1 mm^2 region for mitotic count instead of random selection of 10 high power fields.[27,43] Secondly, whole slide imaging will definitely make the process much more user-friendly with additional application of suitable software.

CONCLUSION

This study brings to light a more detailed evaluation of the lymph node deposits, which might be the need of the hour.

Valuable data come with the assessment of ECS, already proved as a single, independent prognostic factor for OSCC spread, but unfortunately, there are still many parameters to

be considered as it becomes clear by the unaltered mortality and morbidity rates of OSCC. This study puts weight on the proliferation and activity rates of the metastatic deposit present in the lymph nodes.

On the onset, clear insight is available – that the lymph node deposits might be in a state of activity or might be transiently indolent, which becomes a very important parameter to understand and study the further spread of disease. We propose that LMI and PLMR can be valuable predictors for disease progression and spread.

A good correlation was found between ECS and rate and type of mitoses. Similarly, a good correlation was seen with the number of abnormal mitoses predominantly seen in cases of macro-deposits and defaced lymph nodes.

An interesting finding in LMI analysis shows Grade I activity in the primary tumours (70.6%) and a very high number of cases (45%+35%) fell in Grade III and Grade II, when the lymph node metastases were evaluated.

This parameter can best be studied by evaluation of mitotic figures (normal and abnormal) in the metastatic deposits, which has yielded good correlation with other clinical and histological parameters – tumour size, differentiation, POI of invasive edge, and ECS.

Future direction

Large sample numbers should be considered to get clarity on active and indolent lymph node statuses based on which distant metastasis susceptibility can be predicted (low risk/high risk). Such a study can offer us clarity on the requirement of aggressive treatment or further treatment to be considered as a customised treatment protocol for OSCC patients, for example, consideration of radiotherapy (dosage and number of exposures).

Robust studies using AI and whole slide imaging can offer better standardisation in this area of interest, which might allow easier screening, mitotic ratio, and index development for further analysis, improving consistency and reducing inter-observer variability.

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Conflicts of interest

There are no conflicts of interest.

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