Original Article

Understanding the approach to interpretation and challenges in measurement of pathological depth of invasion in oral squamous cell carcinoma: A cross-sectional survey of oral and maxillofacial pathologists in India

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Abstract

Objectives: To understand the approach to interpretation along with challenges encountered in assessing pathological depth of invasion (pDOI) in oral squamous cell carcinoma (OSCC) as per 8th Edition of TNM-AJCC staging among oral and maxillofacial pathologists in India.

Method and Materials: A cross-sectional web-based survey was conducted (May 2021–October 2021) with a pre-validated 21-item questionnaire. Responses were stored in a Microsoft Excel worksheet and analysed by descriptive statistics using SPSS v 25.0.

Results: About 69.7% of the 267 respondents correctly defined pDOI while 13.1% measured the same from tumour surface. Among those not reporting pDOI, one-third of respondents (36.6%) lacked requisite awareness about 8th edition staging while more than half of them (55.4%) lacked proper tools to measure. The vst majority of the oral pathologists found pDOI measurement practically challenging (85.8%), mostly with difficulty in obtaining adjacent normal mucosa (77.9%). Selection of reference points of adjacent normal mucosa was divided between deepest point of rete ridge (43.1%), the closest rete ridge (28.8%) and the tip of highest submucosal papilla (15%).

Conclusion: Underreporting of pDOI was observed owing to inherent challenges in measurement, thus ostensibly substituted with tumour thickness. Elaboration on reference points of adjacent normal mucosa is awaited.

Keywords: Depth of invasion, oral and maxillofacial pathologists, oral squamous cell carcinoma, TNM staging

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Submitted: 02-Jan-2023, **Revised:** 07-Mar-2023, **Accepted:** 10-Mar-2023, **Published:** 12-Sep-2023

INTRODUCTION

The global incidence of oral cancer is estimated at about 300,000 with an annual mortality of 145,000.[1] Oral squamous cell carcinoma (OSCC) constitutes 90% of these with an annual incidence of 1,35,929 in India with

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_1_23

How to cite this article: Rath R, Smitha T, Bhatta A, Nagar SR, Mishra S, Sharma G. Understanding the approach to interpretation and challenges in measurement of pathological depth of invasion in oral squamous cell carcinoma: A cross-sectional survey of oral and maxillofacial pathologists in India. J Oral Maxillofac Pathol 2023;27:481-8.

an overall survival (OS) of less than 50%.[1-3] Current treatment options include surgical resection, radiotherapy, chemotherapy and immunotherapy; the choice of appropriate treatment largely depends on disease stage.[4] The Tumour, Nodes and Metastases (TNM) staging system of the American Joint Committee on Cancer (AJCC) is a simple, universally employed tool that describes the disease extent (stage) and provides prognostic information to aid in formulating therapeutic strategies.[5] There are constant attempts to improve its predictive accuracy by additional histopathological parameters.[6]

The 7th edition of the AJCC staging laid emphasis only on the size and not on the depth of tumour infiltration, thus performing poorly in predicting prognosis in a few early stage OSCCs.[5,6] Microscopic measurement of depth of invasion (DOI) has long been considered a valuable parameter for predicting regional nodal involvement and survival in OSCC.[7,8] The most recent iteration of the TNM-AJCC staging (8th edition) saw major revision with incorporation of DOI as an essential data to be reported for determining the T stage of the primary tumour for OSCC.[5] DOI can be measured clinically and histologically. Guidelines mandate an imaginary line/horizon be drawn across the tumour joining the basement membrane (BM) of normal adjacent intact mucosa on either side and a perpendicular plumb line dropped from the horizon extending to the deepest part of the tumour that would represent the pathological depth of invasion (pDOI).[6] DOI increases pathological T stage (pT) by one step for every 5 mm and leads to stage migration based on 5 mm and 10 mm cut offs.[5,6]

Recent reports have highlighted uncertainties and significant challenges existing in areas pertaining to how pDOI is defined and measured; quite often left to individual interpretation, thus undermining its applicability in the practical scenario.[9,10] Therefore, the present survey attempts to understand the impact of addition of pDOI to 8th Edition (Edn) of TNM-AJCC staging on oral and maxillofacial pathologists in India and their approach to understanding, interpreting and adopting the same in their practice.

MATERIALS AND METHODS

A web-based cross-sectional survey was conducted among oral and maxillofacial pathologists across India, between May 2021 and October 2021. Ethical approval for the survey was obtained from the Institutional Ethical Committee (IEC/SCBDCH/098/2021). The content of the 22-items, self-structured questionnaire was validated by three expert pathologists (2 onco-pathologists and 1 oral and maxillofacial pathologist), using a quantitative index namely (*Aiken's V index*) and reliability was assessed by test-retest reliability method after an interval of 15 days. The final 21 items in the questionnaire with satisfactory content validity scores consisted of close-ended items broadly designed to assess: awareness and interpretation of definition and measurement of pDOI as per AJCC 8th Edn TNM staging [6]; distinction from *tumour thickness* (TT); awareness on requisite grossing technique; choice of tool, interpretation and practical challenges if any in measuring pDOI; awareness about pDOI assessment in frozen sections as well as diagnostic biopsies and impact of pDOI on staging and prognosis.

Google forms were generated and distributed via mail link to a list of 500 oral and maxillofacial pathologists obtained from the Indian Association of Oral and Maxillofacial Pathology *(IAOMP)* database. Reminder emails were sent in case of no response and/or delay in response.

The data collected were exported from Google spreadsheet into Microsoft Excel Worksheet for data mining. Descriptive statistics including frequencies and proportions were computed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

A total of 267 responses were received by the final access date (31/10/2021) of which 128 respondents (47.9%) were attached to teaching institutions, 114 (9.4%) practised exclusively in oral cancer facilities/centres while 25 respondents (42.7%) were involved in both. A total of 227 respondents (85%) reported receiving less than 500 cases annually.

Understanding and employability of DOI; distinction from tumour thickness

A total of 166 respondents (62.2%) claimed to be assessing DOI as per guidelines laid down by AJCC 8th Edn TNM staging. A total of 186 respondents (69.7%) correctly defined pDOI while 35 (13.1%) stated measuring the same superiorly from the tumour surface, which essentially implies the TT. Reasons cited for not reporting pDOI were the lack of proper awareness about the new staging system (36.6%); difficulty encountered in adopting the new criteria (28.7%) and the lack of proper tools to measure DOI (55.4%).

Grossing for DOI

A total of 229 respondents (85.8%) felt it was mandatory to ensure the incorporation of normal mucosa (if available)

adjacent to tumour on both sides for pDOI assessment. A total of 103 respondents (38.6%) felt that tumour should be sampled at every 2–3 mm interval and sections with the maximum depth should be thereafter identified. However, 86 pathologists (32.2%) agreed that a minimum of four sections may be adequate while 65 of them (24.3%) differed that few full thickness sections of tumour with deep margin would suffice. A small margin of respondents felt that the sampling technique did not significantly affect pDOI.

Tools for DOI measurement

Majority of respondents opted for whole slide imaging (52.1%) while others preferred ocular micrometry (25.8%). A total of 30 respondents (11.2%) would measure pDOI using transparent scale on slide under scanner objective while 29 pathologists (10.9%) preferred a technique of dotting the slide with a marker and calculating with a scale outside the microscope.

Interpretation of DOI measurement

In the absence of normal mucosa on one side, 85 respondents (31.8%) chose to extrapolate a line from BM of the opposite side normal mucosa while 25 of them (9.4%) would measure tumour thickness if mucosa is absent on both sides. A total of 157 pathologists (58.8%) responded to adopting both options.

Responses on choosing precise reference points on the BM to draw the horizon varied from the line joining the BM of *deepest point of rete ridge* (43.1%), the *closest rete ridge* (28.8%) or the *tip of highest submucosal papilla* (15%) of adjacent normal mucosa [Graph 1, Figure 1]. A total of 232 pathologists (86.9%) opined that the differential selection of above reference points may cause significant differences in pDOI estimation. Majority of the responses (49.4%) reflected that the shape of the horizon shall depend on natural contour of the uninvolved mucosa but a sizable number of respondents conformed to the horizon being a straight line (41.6%). 24 pathologists (9%) sided with an arcuate horizon. Figure 2 depicts the possibilities of tracing the horizon as straight or arcuate.

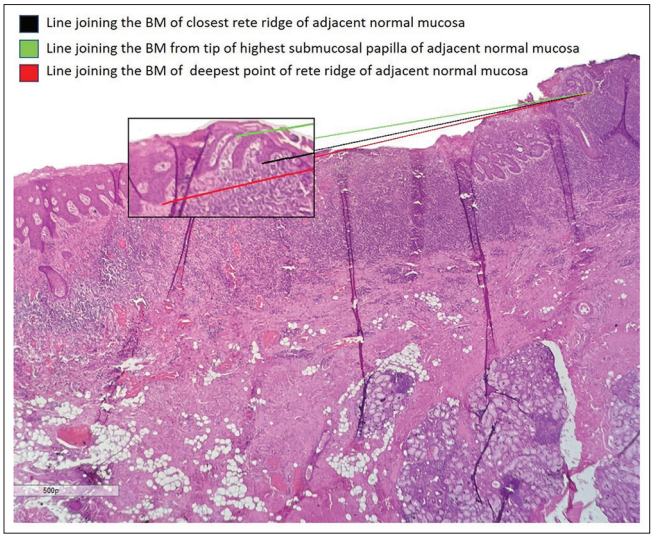


Figure 1: Tracing the horizon from basement membrane of adjacent normal mucosa with three different possibilities of choosing reference points. The red, black and green lines extend from the deepest point of adjacent rete ridge, the closest rete ridge the tip of highest submucosal papilla respectively in the microphotograph (H & E, x2X)

If sections of maximum depth cannot be accommodated in a single slide, 64.8% would measure pDOI in successively cut sections and summate the measurements while 35.2% would prefer to assess DOI subjectively as per anatomical deep structures invaded by tumour.

Challenges in measurement of DOI

A total of 229 respondents (85.8%) found pDOI measurement as per new staging practically challenging due to several reasons [Graph 2, Figure 3]. If facing ambiguity in DOI measurement, 186 pathologists (69.7%) would reportedly substitute it with tumour thickness.

DOI measurement in biopsies

A total of 144 respondents (53.9%) felt it was not feasible to report pDOI in biopsies. A total of 113 of them (78.5%) mentioned the lack of full thickness biopsies as a limiting factor while other pathologists reported factors like the lack of adjacent normal mucosa (47.2%) or fragmented tissue (39.6%) could also pose hindrance.

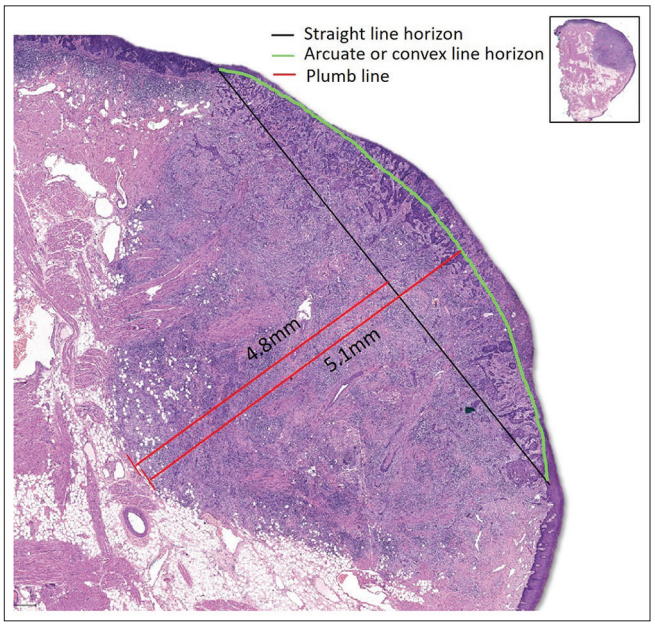


Figure 2: Microphotograph of probable shapes of horizon (H & E, x2X). Variation in DOI measurement manifesting with green arcuate line (pDOI-5.1 mm) and black straight-line horizons (pDOI-4.8 mm) after dropping the plumb line (in red)

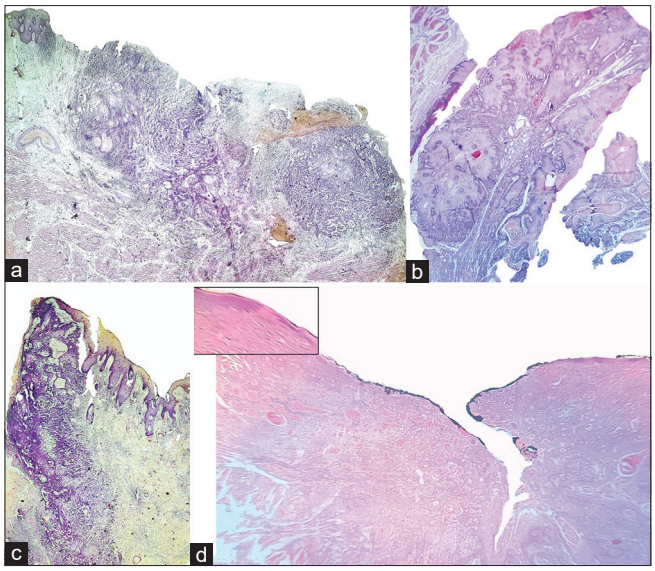
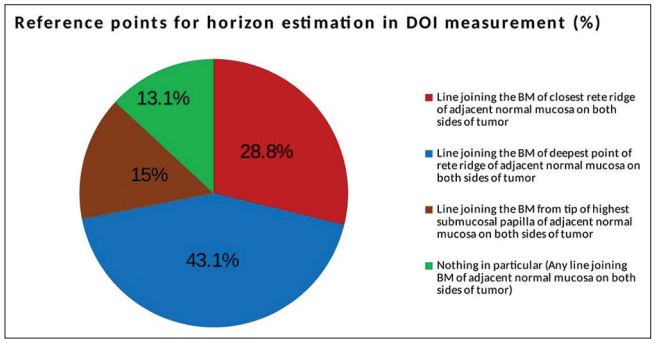
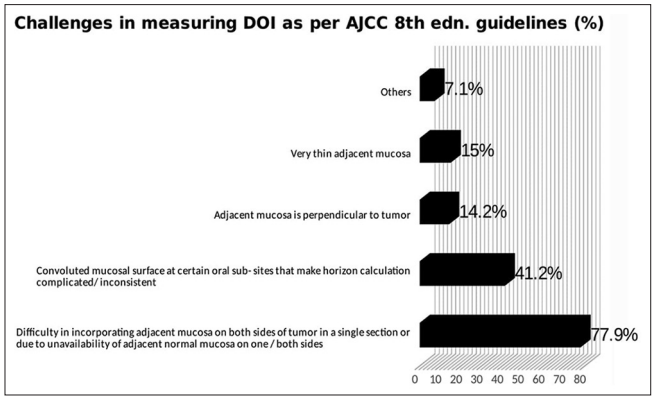


Figure 3: Microphotograph representing the challenges in measurement of pDOI (H & E, x2X). 3a-Lack of adjacent normal mucosa on one side; 3b-Convoluted mucosal surface; 3c-Adjacent normal mucosa perpendicular to tumour; 3d-Thin adjacent mucosa



Graph 1: Pie chart showing the responses to question: How would you choose the reference points for horizon estimation in DOI measurement?



Graph 2: Bar graph depicting responses to question: What according to you could broadly be the causes of challenges in measuring DOI as per AJCC 8th edn. guidelines? (Tick one/more as applicable)

DOI in frozen sections

A total of 159 respondents (59.6%) would recommend formalin fixed paraffin-embedded (FFPE) sections for DOI assessment while 82 pathologists (30.7%) would prefer frozen sections for the same. A total of 26 respondents (9.7%) were unequivocal about choosing either of the two.

Impact on staging and prognosis in OSCC

A total of 255 respondents (95.5%) conformed that DOI had an impact on staging and is of prognostic significance. A total of 201 respondents (75.3%) designated DOI as the most significant histopathological prognosticator in OSCC [Graph 3] followed by lymph node metastasis (58.1%) and tumour grade (41.9%).

DISCUSSION

DOI had started getting analysed with the 6th edition of TNM staging, but its precise definition was clarified as it got incorporated in the 8th edition.[5]

However, there remains ambiguity over clear use of the definition and challenges in the measurement of pDOI.[9,11] A survey was conducted to this effect among oral and maxillofacial pathologists *(who specialise in the diagnosis and reporting of OSCC)* from India. Majority of the pathologists received less than 500 OSCC cases annually despite a high cancer burden in India. Around 69.7% of our respondents correctly defined pDOI. A survey among 184 head and neck surgeons found that 78.9% of their respondents could correctly identify the clinical DOI.[12]

Most of the respondents would slice the tumour at every 2–3 mm interval for pDOI as affirmed in studies, while sampling minimum of four sections at larger intervals is also recommended.[13,14] Since DOI has greater impact on staging of small but infiltrative tumours, full sampling in such tumours at small intervals of 2–3 mm may be recommended while sampling at 10 mm interval with full-thickness sections and the deep margin for large tumours would be feasible.[15]

Current staging scheme for OSCC assigns pathological TNM stage 4 for DOI exceeding 20 mm. Receiver operating curve analysis in a recent study revealed that DOI >20 mm gave the best cut off for OS and relapse-free survival (RFS) and suggested incorporation of the same.[16] Considering maximum depth in deeply infiltrative tumours with pDOI beyond 10 mm may not be accommodated in a single slide, we suggest measurements of successive sections to be summated to calculate the total DOI as affirmed by few authors and gathered in 64.8% of our survey responses.[15]

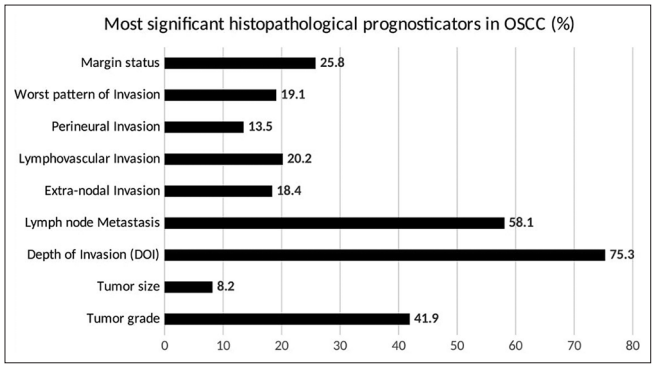
Numerous difficulties in determining the pDOI have been highlighted arising due to uneven BM resulting

from undulating epithelium, curved mucosa and mucosa on multiple sides (tongue), very thin adjacent mucosa or lack of adjacent normal mucosa altogether.[11,17] Similar challenges were reported by 77.9% of our respondents. Kukreja *et al*.,[9] were unable to measure pDOI in 43/95 tumours while unambiguous measurement was possible in only 22.5% of their cases. Incorporation of adjacent normal mucosa may not always be feasible or conversely, the section with adjacent normal mucosa may not include deepest part of the tumour. To circumvent the problem of missing normal mucosa on one or both sides, 90.6% of the respondents would extrapolate a line from adjacent normal mucosa or measure TT respectively which agrees with studies.[11,17]

To determine the horizon, an arch-like line connecting normal mucosa on both sides with the plumb line dropped from its mid-point has been employed rather than strictly considering a straight line as suggested by AJCC; though our responses were divided.[6,11,17] Others have suggested extrapolating to a comparatively straighter area if the BM is undulating.[15]

TT is measured from tumour surface for an exophytic or endophytic tumour and from the ulcer base for an ulcerated tumour to the deepest point of invasion.[18] It is relatively greater than DOI in proliferative tumours and less than DOI in ulcerated tumours [Figure 4].

Though TT is defined by AJCC 7th edition and is reportedly a significant factor in predicting the development of nodal metastasis,[19] it does not find a place in TNM staging.[6] Most studies have used DOI and TT interchangeably, defined the parameters and addressed their measurements inconsistently or measured only one of the variables.[8,20] Past approaches for measurement of DOI have considered either surface line or BM line of surrounding healthy mucosa.[21,22] AJCC 8th edition defined DOI clearly and incorporated it as a pT staging parameter based on data correlating DOI as an independent prognostic factor for disease-specific survival (DSS) from large multi-centre collaborative retrospective study of 3,149 patients of OSCC.[7] Prognostic significance of TT was not addressed and thus excluded. Studies have found DOI, but not TT to be an independent factor in predicting LN metastasis[8,9,14,20] and significantly associated with locoregional recurrence,[13] DSS[13,23] and progression-free survival.[23] A recent meta-analysis found higher DOI to have greater chance



Graph 3: Bar graph depicting responses to question: What according to you are the most significant histopathological prognosticators in OSCC? (Tick three options)

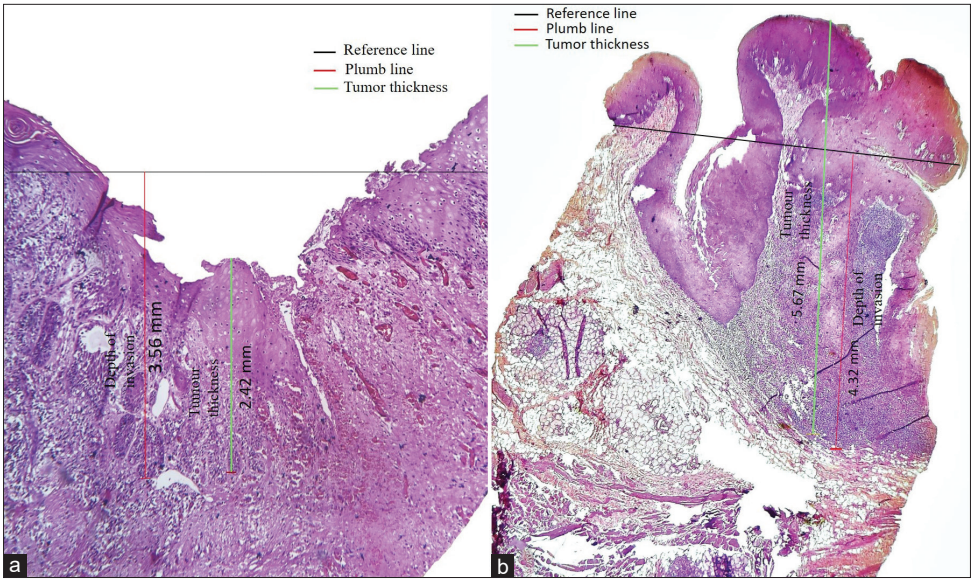


Figure 4: Measurement of DOI and TT in OSCC. 4a-In ulcerated tumour (TT measured from surface of ulcer base is smaller (2.42 mm) in comparison to pDOI (3.56 mm), 4b-In exophytic tumours (TT measured from tumour surface exceeds (5.67 mm) pDOI (4.32 mm) in shown in microphotograph (H & E, x2X)

of LN metastasis, recurrence and lower survival, corroborating its incorporation for OSCC staging.[24] Others have found both TT and DOI to carry comparable prognostic significance.[25,26] Liu *et al*.[26] found DOI and TT to be highly correlated with nodal risk but a 4.5 mm cut point of DOI to be superior than the 8 mm cut point of TT for prediction of nodal disease, also stressing on the need to recognise both parameters as discreet entities. Salama *et al*.[17] reviewed 293 tongue OSCC and found both DOI and TT performing identically in predicting OS and LN metastasis with high correlation between the parameters (0.984), thus proposing to replace DOI by TT. 69.7% of our respondents preferred to substitute with TT when facing ambiguity in DOI measurement.

Most authors in developed countries use whole slide imaging and carry out precise pDOI measurements.[23] AJCC recommends ocular micrometry but few authors have questioned its availability in resource-limited setting like India.[6] Ocular micrometry requires multiple movements of stage to capture entire depth at times or the use of 2× scanner objective and eyepiece. Few authors use clear plastic ruler to assess pDOI by viewing it under 2.5× scanner objective.[9,11] For pDOI exceeding 10 mm, corroboration has also been done by placing dots on the slide and measuring with a scale.[9] Most of our respondents conform with use of whole slide imaging while sizable responses were in favour of ocular micrometry.

Around 86.9% of our respondents have inferred that in irregularly hyperplastic epithelium, the choice of precise point to trace horizon could impact pDOI. Around 13.1% of our respondents preferred the tip of the highest submucosal papilla as few others,[9] while most opted for the deepest point of adjacent rete ridges. Lee *et al*.[23] overcame comparable limitations by repeating DOI measurements for each slide three times from different points of adjacent rete ridges and using the mean value as final value. The interspersed invasive carcinoma between upper and lower limits of BM may be disregarded if the deepest point of hyperplastic rete ridge is considered; thus underestimating actual DOI.[17] Current staging scheme does not elaborate on these conjectures.

Theoretically though pDOI measurement on full thickness biopsies is a possibility, it is practically impossible if normal mucosa is absent, tissue is fragmented or not truly representative which is invariably noted.[10,12,27] Conversely, 20% of tongue OSCC pDOI needed measurement on diagnostic biopsies as the entire tumour was removed in biopsy with absent/minimal carcinoma in excision sample.[11] Measurement of DOI in diagnostic biopsies and documenting the factors limiting the same needs further study.

Around 95.5% of our respondents felt DOI impacted staging and 75.3% agreed that DOI is the most significant prognosticator. Wunschel *et al*.[28] found DOI to be the strongest histological predictor of LN metastasis, OS and RFS of OSCC patients among other parameters. As DOI may fall short in predicting LN metastasis and DSS if used as the only prognostic parameter, prediction models and nomograms however that have factored in a variety of other clinically relevant and histopathological variables have performed well and might obfuscate the overemphasis of DOI or tumour size alone particularly in distinguishing T1 and T2N0 OSCC.[20,29] Studies have identified factors like grade, perineural invasion (PNI), lymphovascular invasion, tumour budding and worst pattern of invasion to be associated with a higher risk of recurrence and poor survival.[30-32] Subramaniam *et al*.[33] has shown incorporation of PNI ($P = 0.032$) and differentiation ($P = 0.009$) to current edition of TNM staging better reflected OS in early-stage OSCC with suggestions for addition of these parameters for further prognostication. Advocates of DOI point at the qualitative nature of many of the histopathological parameters, prone to subjectivity bias while DOI is measured quantitatively and therefore more precise.

Kane *et al*.[14] hypothesised that while other parameters are practically difficult to assess in frozen sections, DOI can be evaluated nonetheless an optimal cut off needs to be devised. Around 30.7% of our respondents recommended DOI assessment in frozen sections over FFPE sections. S Kumar *et al*.'s[34] study has evaluated accuracy of DOI in frozen sections and found a high correlation with DOI in permanent hematoxylin and eosin stained sections.

CONCLUSION

Results of our survey shed light on the awareness and understanding of pDOI among oral and maxillofacial pathologists in India. There seems to be underutilisation of the current staging system, which may have an impact on patient treatment and outcome. To heed suggestions of using a more simplified parameter like TT, evidence-based studies comparing prognostic ability of TT and DOI in large-scale cohorts must come up. Currently, *TT may suffice as an alternative to DOI* in cases where practical challenges cannot be overcome, more so in a *resource poor setting*. There seems to be little doubt that tissue specimens need to fulfil certain minimum standards to be evaluated but there is a need for clear and meticulous interpretation of definition of DOI and its measurement in biopsies.

Our recommendation is systematic multi-centre prospective studies investigating the effect of different approaches in measurement of DOI (*drawing of horizon from different points on BM of adjacent normal mucosa*) on LN metastasis and prognosis. Our survey may have design limitations of not generating objective information and accessing only those members who are registered in IAOMP database.

Acknowledgements

We would like to acknowledge with gratitude the time and expertise rendered by Dr. Swapnil U Rane [M.D., D.N.B., P.D.C.C., MRes (TCM)], Associate Professor (E) of Pathology, Tata Memorial Centre (TMC), Mumbai, India. Dr. Manas Baisakh [MD (Path); PDCC (Oncopath)], Sr. Consultant Histopathologist, Apollo Hospitals, Bhubaneswar, Odisha, India and Dr. Jyoti Bhavthankar (MDS, Oral and Maxillofacial Pathology), Professor and Head of Department of Oral and Maxillofacial Pathology, Govt Dental College and Hospital, Aurangabad, Maharashtra, India, in validation of the questionnaire content.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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