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Radiologic depth of invasion as a predictor of cervical nodal metastasis in oral cavity squamous cell carcinoma: A retrospective cohort study

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Research Article

Keywords: depth of invasion, nodal stage, occult nodal metastasis, pathological DOI, radiological DOI, surrogate marker

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Title Page

Title: Radiologic depth of invasion as a predictor of cervical nodal metastasis in oral cavity squamous cell carcinoma: A retrospective cohort study

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Manuscript Abstract

Title: Radiologic depth of invasion as a predictor of cervical nodal metastasis in oral cavity squamous cell carcinoma: A retrospective cohort study

Abstract:

Purpose - Depth of invasion (DOI) and nodal stage are important prognostic factors of oral cavity cancer. Accurate determination of DOI depends on pathology assessment after surgery. We planned to assess correlation between radiological DOI (rDOI), pathological DOI (pDOI) and pathological nodal stage (pN).

Methods - This is a retrospective analysis of 560 patients with oral cavity cancer treated with radical intent from January 2017 to December 2021. rDOI was measured by two radiologists independently and correlated with pDOI. Relation between DOI and nodal stage was investigated.

Results - We observed very strong inter-reporter reliability for rDOI's measured independently. rDOI significantly correlated with pDOI (Spearman's rho = 0.916 and p-value<0.0001). Further, there was significant positive correlation between pDOI and pN (rho-0.337 and p<0.0001). Among patients with pDOI <1 cm, only 39% had nodal involvement, compared to 67% in the ≥1 cm group. Also, patients having pDOI >1cm had twice the incidence of pN3b.

Conclusion - Measuring rDOI is feasible and comparable to pDOI and a predictor of pN stage and occult metastasis in clinically node negative patients. rDOI may be used as a reliable surrogate for planning nodal dissection in oral cavity cancer.

Keywords: depth of invasion, nodal stage, occult nodal metastasis, pathological DOI, radiological DOI, surrogate marker

Introduction:

Oral cancer is the sixth most common cancer worldwide accounting globally for 377,713 new cases in 2022, which represents an increase in incidence from 2018. Oral cancer is a major problem in the Indian subcontinent with age-adjusted rates of oral cancer as high as 20 per 100,000 population and accounts for over 30% of all cancers in the country. The 5-year overall survival remains dismal, ranging from 70% in early stage oral cavity cancer (OCC) to 27% in advanced stage OCC [1,2].

Nodal stage and depth of invasion (DOI) are important prognostic factors of OCC, assessment of which will guide our treatment plan. Some nodes may appear normal but have occult metastasis. DOI refers to the distance between the level of the basement membrane relative to the closest intact squamous mucosa and the deepest point of invasion of the carcinoma. So, accurate assessment of DOI can only occur on pathological evaluation after surgery. Importance of DOI can be understood by it's inclusion in oral cavity cancer staging by American Joint Committee on Cancer (AJCC) 8th edition where higher DOI upstages the primary tumour stage indicating higher chances of spread and poorer outcomes. DOI can be presumed radiologically (rDOI) using imaging modalities, which can be used for preoperative non-invasive assessment [3].

Hence, we planned to conduct a retrospective analysis of patients with oral cavity cancer who were treated with radical intent with surgery and adjuvant radiation/ chemoradiation, if indicated, to assess correlation between rDOI and pathological DOI (pDOI) and between pDOI and occult nodal metastasis and nodal stage to understand whether rDOI can be used preoperatively as surrogate for pDOI and nodal stage.

Methods:

Patients diagnosed with biopsy proven OCC who were treated with radical intent and had pre-operative radiological imaging with CECT or MRI of face and neck from January 2017 to December 2021 at a tertiary care oncology centre in India were considered for retrospective analysis. The study protocol was approved by the institutional ethics committee and institutional review board. Informed consent was taken from patients for using images and pathological data.

Patients with metastatic OCC, recurrent tumour, preoperative chemotherapy or radiotherapy, incomplete surgery, revision surgeries and non-squamous histology were excluded.

All patients of OCC were discussed in multidisciplinary tumour board. Demographic, clinical, and pathological data of 342 patients of OCC who were managed with surgery as the primary mode of treatment with radiation or chemoradiation, as indicated, were retrieved from hospital records. Radiological assessment was done with either CECT or MRI of face and neck. rDOI was measured by two radiologists independently, as rDOI-1 and rDOI-2. pDOI and nodal stage (pN) was recorded from final pathology report. DOI was stratified into two groups - <10mm and >10mm and compared.

Data obtained was compiled and analysed in IBM SPSS statistics 23.0 (IBM Corp., Armonk, NY, USA). Frequency tables and descriptive statistics were used to present demography, tumour and treatment characteristics. Intra-class correlation was used to assess correlation between rDOI-1 and rDOI-2. Shapiro-Wilk test was done to confirm normal distribution of data. As the data was not normally distributed Spearman's correlation test was done to assess correlation between rDOI and pDOI. Correlation between pDOI and pN was assessed using Spearman Rank Correlation test. Also, pDOI was correlated with nodal stage and relation with occult nodal metastasis. Receiver Operating Characteristic (ROC) analysis was used to find optimal cut-off for nodal metastasis and occult pathological nodes. rDOI was correlated with nodal stage and ROC analysis was done.

Results:

Patient and tumour characteristics

Through assessment of records, 560 patients were identified as OCC. Of these, 342 patients were found suitable and were included in this study. Demography and tumour characteristics are summarised in Table 1. Mean age was 55.1 years and OCC was 6 times more frequent in males. Chewing tobacco was the most common form of addiction as observed in 28% cases. The most common site of tumour was buccal mucosa (41.8%) followed by gingivo-buccal sulcus (34.8%). (Table 1) Squamous cell carcinoma was the most common histology observed (98.2%). Majority of the patients presented in locally advanced stage at the time of diagnosis. (Fig. Ia & Ib) Mean rDOI was 1.51cm whereas mean pDOI was 1.34cm. Post-

operative histopathology revealed pathological nodes in 58% patients and extra-nodal extension (pENE) in 42.4% cases.

Inter-reporter reliability of rDOI-1 and rDOI-2

rDOI was measured by 2 radiologists independently. Concordance between rDOI-1 and rDOI-2 was assessed for 120 patients. Two-way mixed intra-class correlation was 0.966 (95% CI - 0.95 - 0.97) with excellent reliability.

Correlation between rDOI and pDOI

rDOI and pDOI was assessed in 279 patients using Spearman's correlation. There was a statistically significant very strong correlation between rDOI and pDOI with Spearman's rho = 0.916 and p-value<0.0001 indicating radiological measurement of DOI is representative of pDOI.

Correlation between pDOI and pN

Spearman Rank Correlation test was used to assess correlation between pDOI and pN in 339 patients. There was a moderate, statistically significant positive correlation between tumour depth and nodal stage (rho-0.337 and p<0.0001) suggesting that deeper tumours are associated with more advanced nodal stage. Kruskal Wallis test was used to confirm whether advanced nodal stage will have deeper tumours. There was a statistically significant difference in pDOI across pN stage (Chi square - 40.56, p<0.0001) indicating DOI differs across nodal stage. (Fig. IIa) However, for pairwise comparison of pDOI across pN stage using Mann-Whitney U test with Bonferroni correction (p value = 0.0024), the difference was statistically significant only for N0 and N3b stage (U= 4785 and p= 0.0005). The probability of nodal metastasis across DOI is outlined in Fig. IIIc.

Ordinal regression showed that DOI is a strong, statistically significant predictor of pN stage.(Fig. IIIa) Every mm increase in DOI increases the odds of having a more advanced pN stage by about 2.4 times (95% CI - 1.8x - 3.2x). In multivariable ordinal logistic regression, pDOI (Odd's ratio (OR) =1.59, 95% CI: 1.13-2.25, p=0.008), tumour differentiation (OR =2.47, 95% CI - 1.16–5.26, p = 0.019) and pathological T stage (OR =1.45, 95% CI: 1.07–1.97, p = 0.018) were significant independent predictors of nodal stage. Age, gender, subsite and clinical primary tumour stage were not statistically significant. Receiver Operating Characteristic (ROC) analysis was done to determine the optimal DOI cut-off value to predict nodal metastasis. DOI of 0.95cm showed sensitivity of 79.2% and specificity of 44.8%. Area under curve (AUC) was 0.679 (95% CI: 0.621–0.736, p < 0.001). (Fig. IVa, Va)

Effect of stratified pDOI on nodal metastasis

pDOI was stratified into two groups: <1cm and ≥1cm. Ordinal logistic regression was performed to assess association between stratified pDOI and pN. Patients with pDOI ≥ 1 cm had significantly higher odds of nodal metastasis (OR = 3.04, 95% CI: 1.94–4.77, $p < 0.001$) compared to those with DOI < 1 cm. This finding supports the clinical relevance of using a 1 cm pDOI threshold as a predictor for nodal involvement. Also, among patients with pDOI <1 cm, only 39% had nodal involvement, compared to 67% in the ≥1 cm group, supporting the use of 1 cm as a clinically meaningful cut-off.

pDOI correlation with occult pathological nodes

In the subset where patients were cN0 (n=143), pN + was observed in 30% (n=44). Tumours with DOI ≥ 1cm had 1.5 times of having nodal metastasis than if DOI <1cm. Effect of pDOI was assessed using binary logistic regression. It was observed that increasing DOI was a significant predictor of occult nodal metastasis in clinically node-negative patients. (Fig. IVb) For each 1 mm increase in DOI, the odds of nodal positivity increased by 2.76 times ($p = 0.001$). It remained statistically significant on multivariable binary logistic regression (OR=2.76, p=0.028). However, other variables including age, gender, clinical primary tumour and nodal stage, differentiation and subsite were not significantly associated with nodal involvement. On Mann–Whitney U test it was found that occult nodal metastasis had significantly higher pDOI than those without nodal involvement among clinically node-negative patients ($U = 1414$, $Z = -3.419$, p=0.001). (Fig. IIb)

ROC analysis was done to determine the ability of DOI to predict occult nodal metastasis. AUC was 0.679 with moderate discriminative ability (p=0.001, 95%CI- 0.58-0.77). (Fig. IIIb) DOI of 0.85cm showed sensitivity of 72% and specificity of 49% to predict occult nodal metastasis in clinically node negative patients while a cut-off at 1.05cm had sensitivity of 54.5% and specificity of 67.0%. (Fig. Vb)

rDOI and pN correlation

As predictive validity needs direct testing, ordinal logistic regression was done to confirm if rDOI can predict pN similar to pDOI. It was concluded that higher rDOI was significantly associated with pN stage with an odds of having 2.36 times higher nodal stage for every 1mm increase in rDOI (95% CI-1.7x-3.3x, p=0.0004).

Discussion:

DOI is considered to reflect outcomes of oral cavity cancer. De-escalation of surgery depending on DOI is currently evolving and is not a standard practice. Also, accurate determination of DOI is possible on pathological evaluation after surgery. Assessment of DOI on radiological imaging (rDOI) prior to surgery can help us plan surgery in an improved way and accurately plan nodal dissection or avoid unnecessary nodal dissection.

In our study, rDOI was measured by two radiologists independently to exclude any error or bias. As the inter-observer correlation was very high confirming the reliability of rDOI, we planned to correlate it with pDOI available from final pathology report. It was observed that rDOI and pDOI had very strong correlation. So, rDOI, as measured on pre-operative imaging, can indicate pDOI. Studies have shown correlation between rDOI and pDOI with pre-operative intraoral ultrasound (USG), MRI and CT as 0.91, 0.85 and 0.82, respectively. We observed a correlation coefficient of 0.917 using CT and MRI diagnostic tests, which was better than that reported in literature. A few studies have reported ultrasound to have slightly improved correlation than MRI and CT due to its ability to better differentiate tumour and related changes, however, as intraoral USG is not a routine part of workup of oral cavity cancer, adding another investigation would increase burden on the patient [4].

This study highlights a strong correlation between rDOI and pDOI which supports the reliability of radiological imaging for pre-operative assessment of DOI. Our results are better than that reported in studies available in literature [5]. However, it must also be noted that slight underestimation or overestimation can occur due to inflammation, motion and changes associated with biopsy. Time interval between imaging and surgery can also affect the results.

Various studies have reported prognostic impact of DOI on OCC patients [6]. Also, OCC presenting with nodal metastasis portends dismal prognosis. So, we planned to correlate pDOI and pN. In our study, there was a significant positive correlation between pDOI and pN. Also, patients with advanced nodal disease tended to have deeper tumours.

Majority of OCC patients in India present in locally advanced stage. Mean and median pDOI in our cohort was 1.3cm and 1.2 cm, respectively. So, we chose 1cm as cut-off value to assess the effect of stratified pDOI on nodal metastasis. Patients with pDOI $\geq$1cm had 1.7 times risk of nodal metastasis than if pDOI was lower than 1cm. Also, patients having pDOI >1cm had twice the incidence of pN3b nodes.

It is known that small nodes with occult metastasis can be difficult to identify on imaging. Our findings show that DOI can be used to predict occult nodal metastasis. ROC showed cut-off value for DOI as 0.85cm. This threshold can be used to better differentiate occult nodes and plan their further treatment accordingly. Studies have reported various cut-off values for DOI ranging from 4-10mm [7,8]. We believe our result is towards higher value due to patients presenting in locally advanced stage. Some authors also suggest that as the risk increases with each mm rise in DOI, there is likely no critical DOI that prognostically indicates nodal metastasis [9]. Similar to the study by Joshi et al, we could not find association of lymph node metastasis with other factors (clinical and pathological T stage, subsite, age, gender and differentiation) [8].

Various prognostic factors have been identified for OCC, yet spread to regional nodes is unpredictable and there is no consensus for threshold for DOI. Through this study, we want to show that 'rDOI' as observed on imaging with CT and MRI, which is a routine part of workup of OCC, can be used as a surrogate to plan nodal dissection. However, there are a few limitations in this study. As it was a retrospective study, the data is susceptible to have inaccuracy in the medical records. Due to resource limitation, some patients had CT as their pre-operative imaging which is less sensitive than MRI to measure DOI. A prospective study and a larger cohort of patients is needed to validate the results.

Conclusion:

Radiological DOI is significantly correlated with pathological DOI. A positive correlation was observed between DOI and nodal stage as well as occult nodal metastasis. For each mm increase in DOI, the odds of having a more advanced pN stage and occult nodal metastasis increases. This study highlights that rDOI is an important prognostic factor and can be used as a reliable surrogate for nodal staging. Thus, rDOI can assist us in planning surgery. Further prospective studies are needed to establish universal DOI cut-offs and explore its integration with treatment guidelines.

Abbreviations: OCC - oral cavity cancer, rDOI - radiological depth of invasion, pDOI - pathological depth of invasion, pENE - pathological extra-nodal extension, AJCC - American Joint Committee on Cancer, OR - Odd's ratio, CI - confidence interval ROC - Receiver Operating Characteristic, AUC - area under curve, USG - ultrasound

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Declarations: This study was presented as a poster in ESTRO 2025.

Ethics approval and consent to participate - All procedures were performed in compliance with the Declaration of Helsinki. This study was approved by the Institutiond Ethics Review Committee and obtained informed consent from the participants.

Consent for publication – Not applicable

Data availability- The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Competing interests - The authors declare no competing interests.

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Authors' contributions – All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Ankur Dwivedi, Dr. Pragyat Thakur and Dr. Deepander S Rathore. The first draft of the manuscript was written by Dr. Pragyat Thakur and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figure Legend:

Fig. (Ia) - Distribution of oral cavity cancer cases based on clinical T stage and pathological T stage; (Ib) - Distribution of oral cavity cancer cases based on clinical N stage and pathological N stage

Fig. (IIa) - Boxplot depicting distribution of pDOI across different pN stages; (IIb) - Boxplot depicting distribution of pDOI across patients with cN0 without and with occult metastasis

Fig. (IIIa) - Plotted logistic curve showed a steep rise in probability with DOI beyond approximately [~0.75cm], suggesting a potential clinical cut-off for nodal metastasis.

(IIIb) - Plotted logistic curve showed a steep rise in probability with DOI beyond approximately [~0.75 cm], suggesting a potential clinical cut-off for occult nodal metastasis (IIIc) - Predicted probability of nodal stage depending on DOI

Fig. IV - Line chart showing the relationship between (IVa) DOI and pN stage (excluding N2c and N3a due to less number of cases) (IVb) DOI and risk of occult nodal disease increases with DOI

Fig. V - ROC curve plotted to evaluate the ability of DOI to predict (Va) - nodal metastasis. AUC was 0.679 (95% CI: 0.621–0.736, $p < 0.001$). ; (Vb) - occult nodal metastasis (pN+) among cN0 patients. AUC was 0.679 (p=0.001, 95%CI- 0.58-0.77).

Table Legend:

Table I - Demographic and tumour characteristics of oral cavity cancer patients

Table I: Demographic and tumour characteristics of oral cavity cancer patients	
Characteristic	**Number of patients**
Age (Mean +/- SD)	55.17 +/- 12.48 years
Gender (Male: Female)	6:1
Addiction: **Yes** **No** **Unknown**	 212 (62%) 64 (18.7%) 66 (19.3%)
Addiction Subtype: **Alcohol** **Smoking** **Tobacco** **Mixed** **None**	 7 (2%) 26 (7.6%) 96 (28%) 83 (24.3) 63 (18.4)
Primary subsite: **Lip** **Tongue** **Gingivo-buccal sulcus** **Buccal mucosa** **Hard Palate** **Floor of mouth** **Alveolus**	 6 (1.8%) 22 (6.5) 119 (34.8%) 143 (41.8%) 5 (1.5%) 5 (1.5%) 40 (11.7%)
Group Stage (AJCC 8th ed) **1** **2** **3** **4a** **4b**	 28 (8.2%) 34 (9.9%) 51 (14.9%) 108 (31.6%) 117 (34.2%)
Mean tumour size (pathological) (cm)	3.5 +/- 1.66
Mean nodal size (pathological) (cm)	1.7 +/- 1.16
Radiological extranodal extension	155 (45.3%)
Pathological extranodal extension	145 (42.4%)
Lymphovascular invasion	83 (24.3%)
Perineural invasion	159 (46.5%)
Margin: **Positive** **Negative** **Close** **Missing**	 10 (2.9%) 293 (85.7%) 36 (10.6%) 3 (0.9%)
DOI r1 (cm) **Mean** **Median** **Range**	 1.51 +/- 0.76 1.4 0.4 to 4.8

Table I: Demographic and tumour characteristics of oral cavity cancer patients	
Characteristic	**Number of patients**
DOI r2 (cm) **Mean** **Median** **Range**	 1.58 +/- 0.77 1.5 0.4 to 4.9
pDOI (cm) **Mean** **Median** **Range**	 1.34 +/- 0.8 1.2 0.1 to 5

286

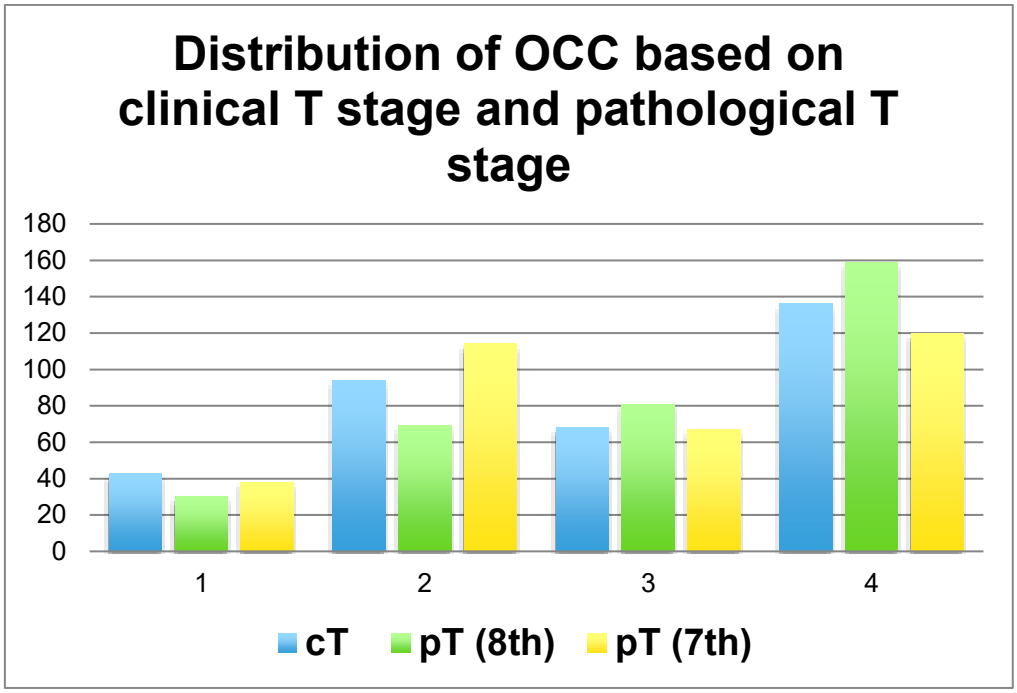
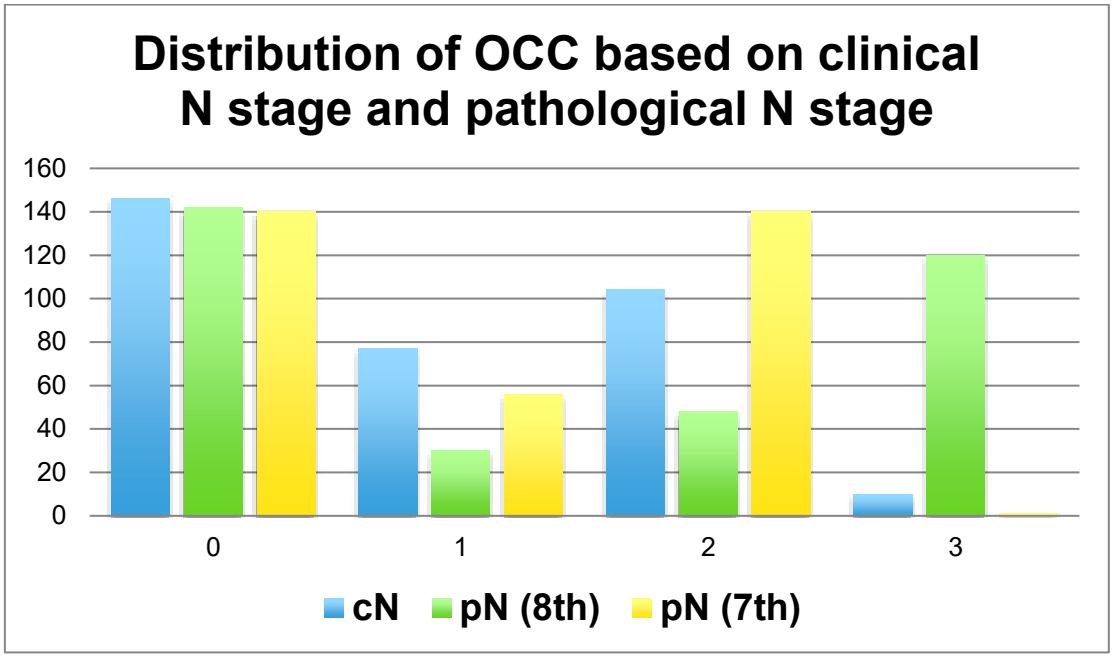
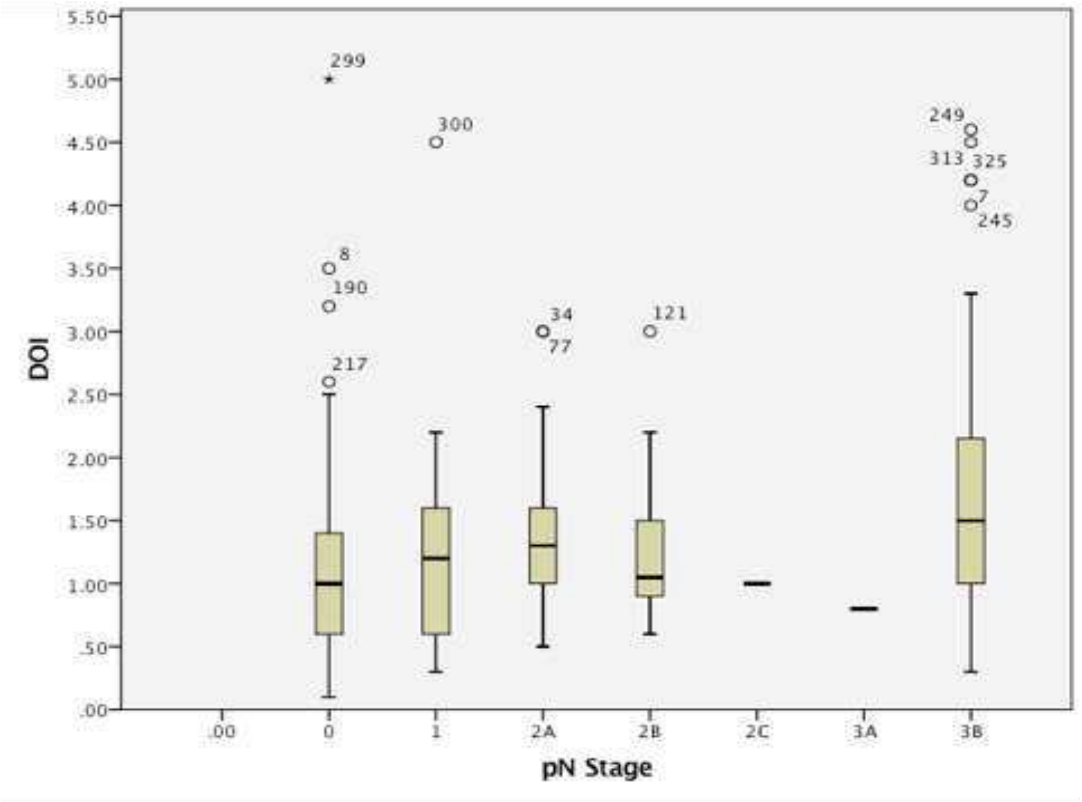

Distribution of OCC based on clinical T stage and pathological T stage
180
160
140
120
100
80
60
40
20
0
1
2
3
4
cT
pT (8th)
pT (7th)

Fig. Ia

289

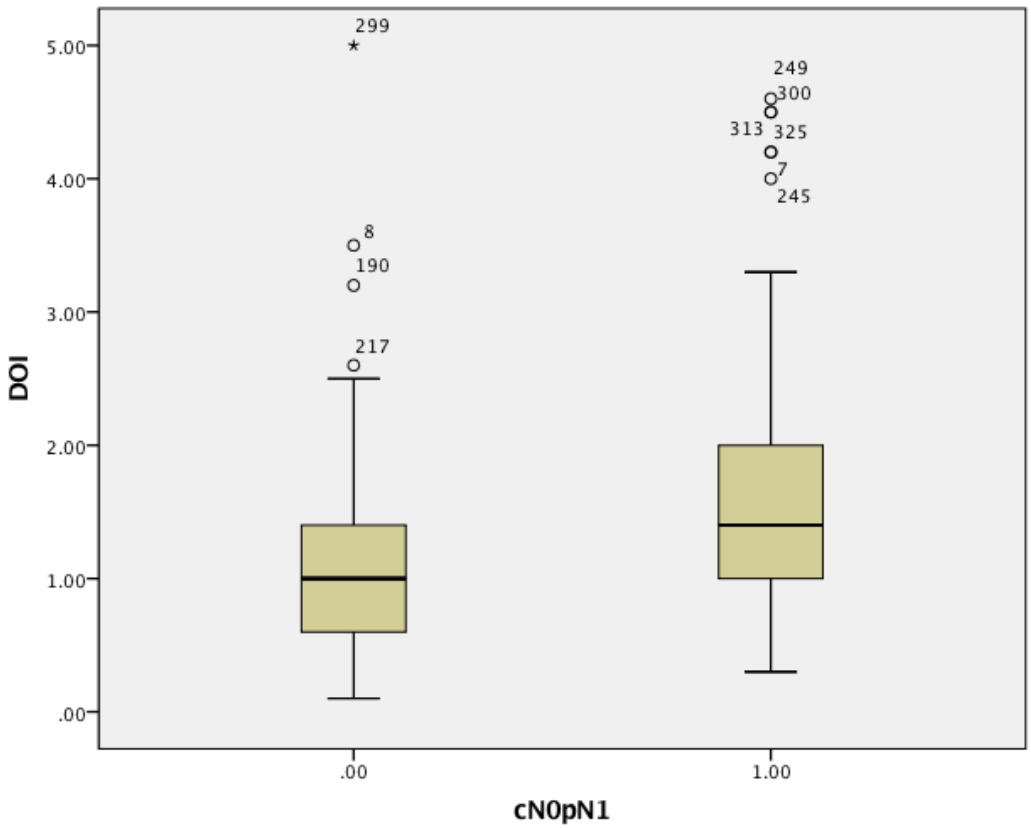


290
291 Fig. Ib



Boxplot depicting distribution of pDOI across different pN

Fig. IIa



Boxplot depicting distribution of pDOI across patients with cN0 without and with occult metastasis

Fig. IIb

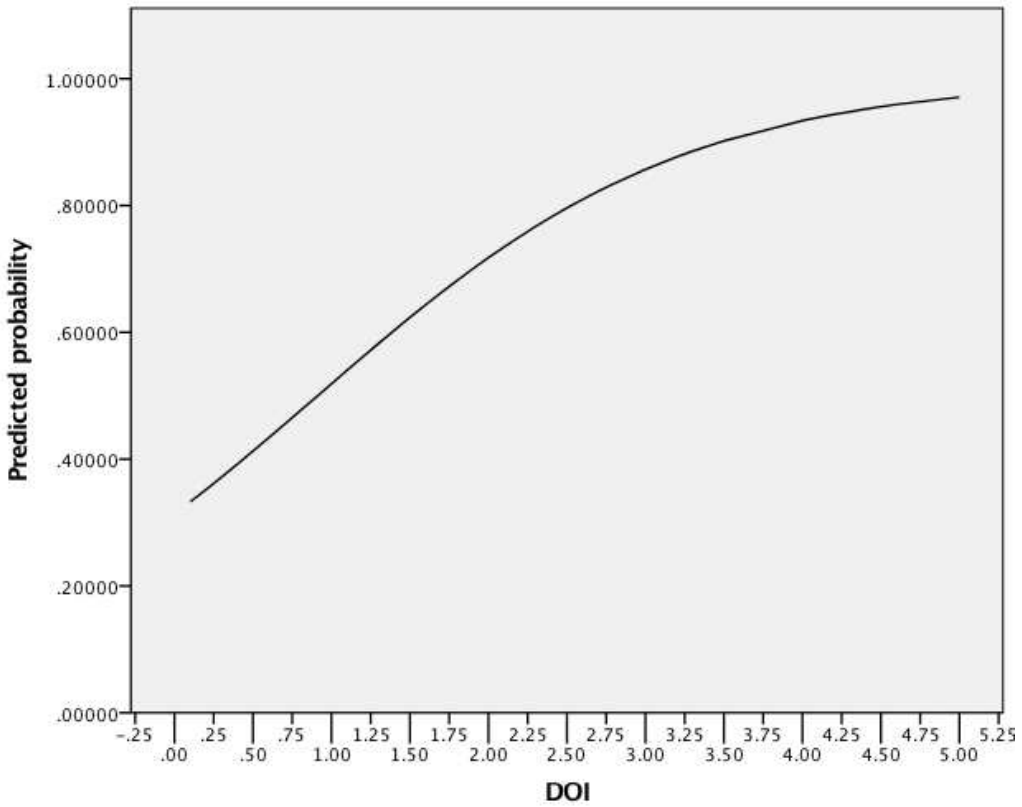

Predicted probability
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.80000
.60000
.40000
.20000
.00000
-.25
.00
.25
.50
.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
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3.75
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4.25
4.50
4.75
5.00
5.25
DOI

Fig. IIIa

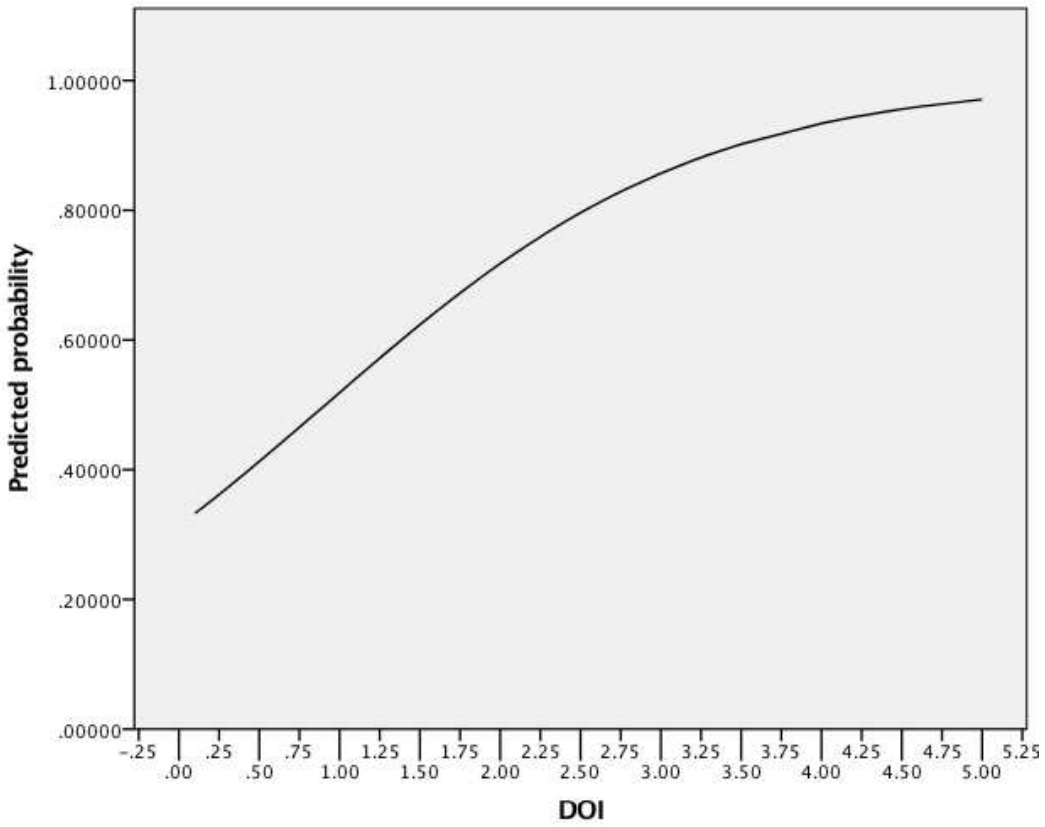
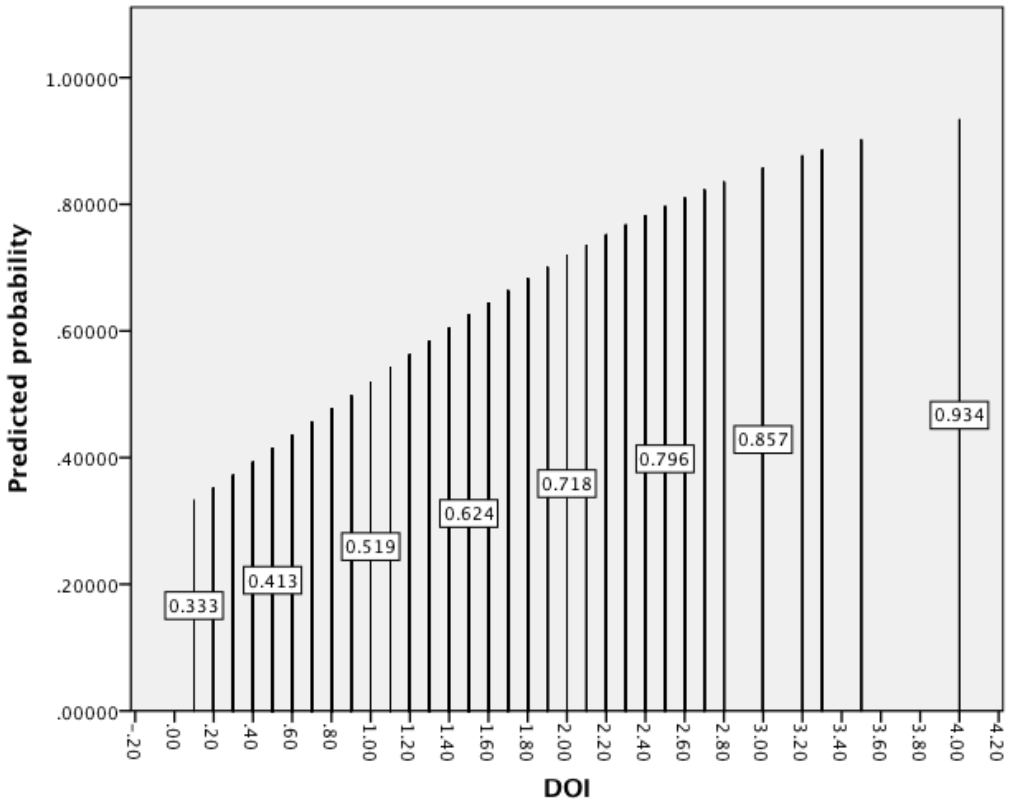

Predicted probability
1.00000
.80000
.60000
.40000
.20000
.00000
-.25
.00
.25
.50
.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
3.50
3.75
4.00
4.25
4.50
4.75
5.00
5.25
DOI

Fig. IIIb

300



301
302 Fig. IIIc

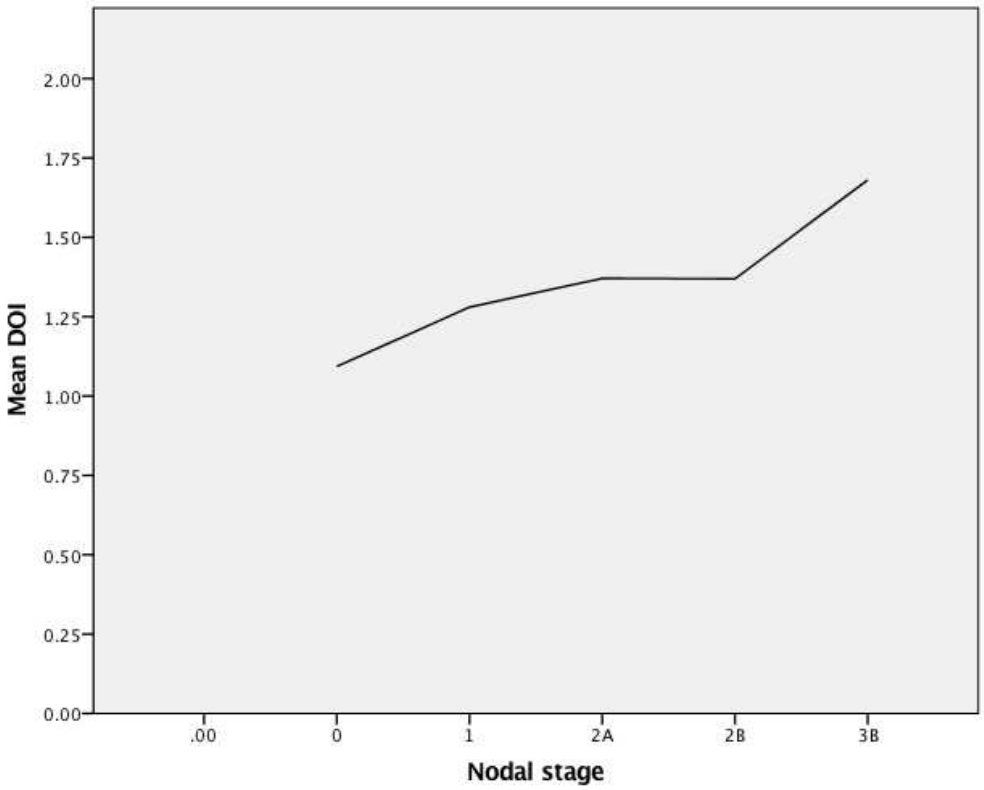


Fig. IVa
Mean DOI vs nodal stge (excluding N2c and N3a due to less no. of cases)

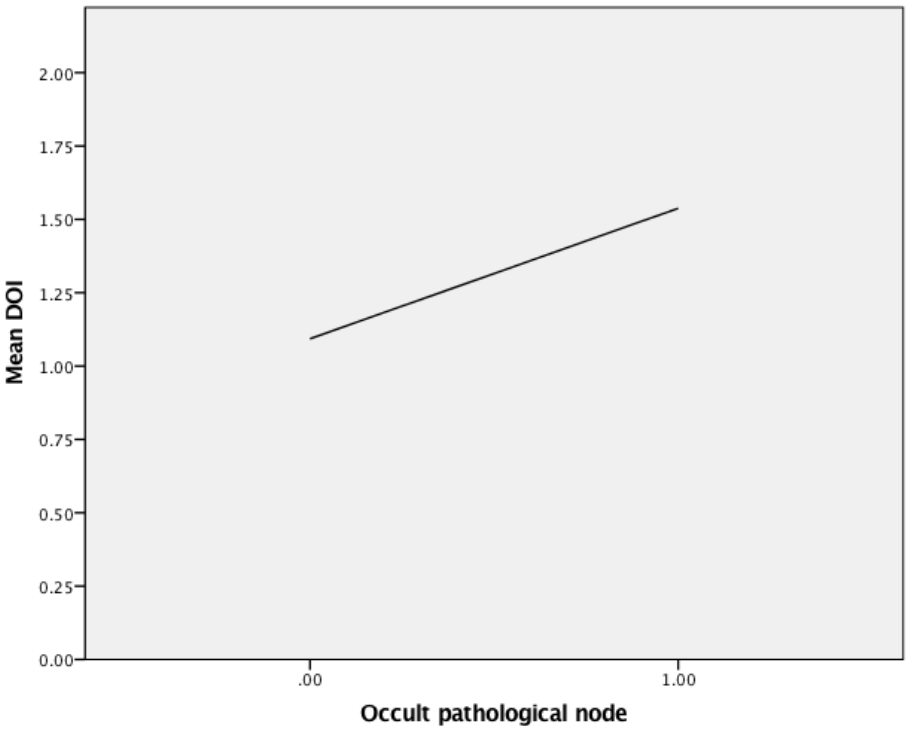

2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Mean DOI
.00
1.00
Occult pathological node

Fig. IVb

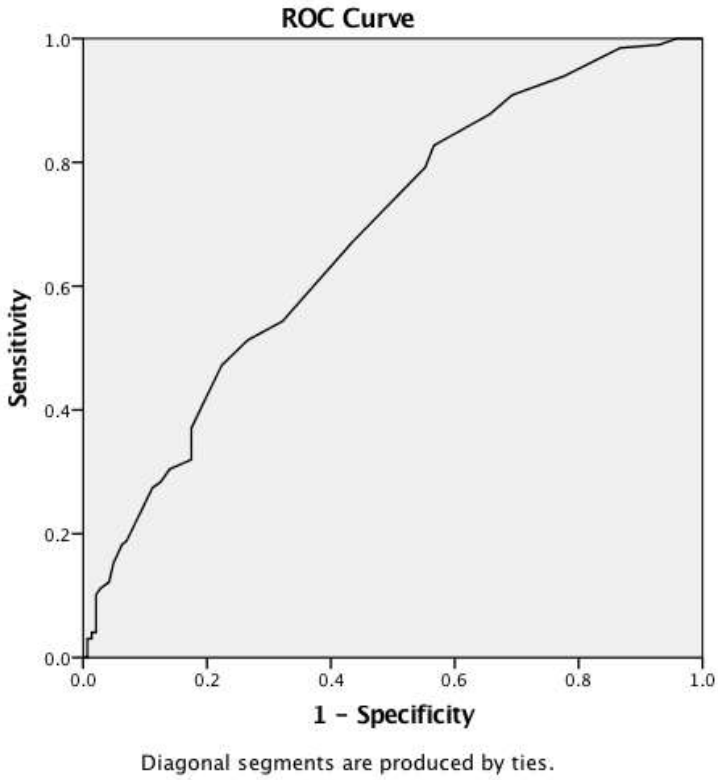

ROC Curve
Sensitivity
1 – Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.

Fig Va

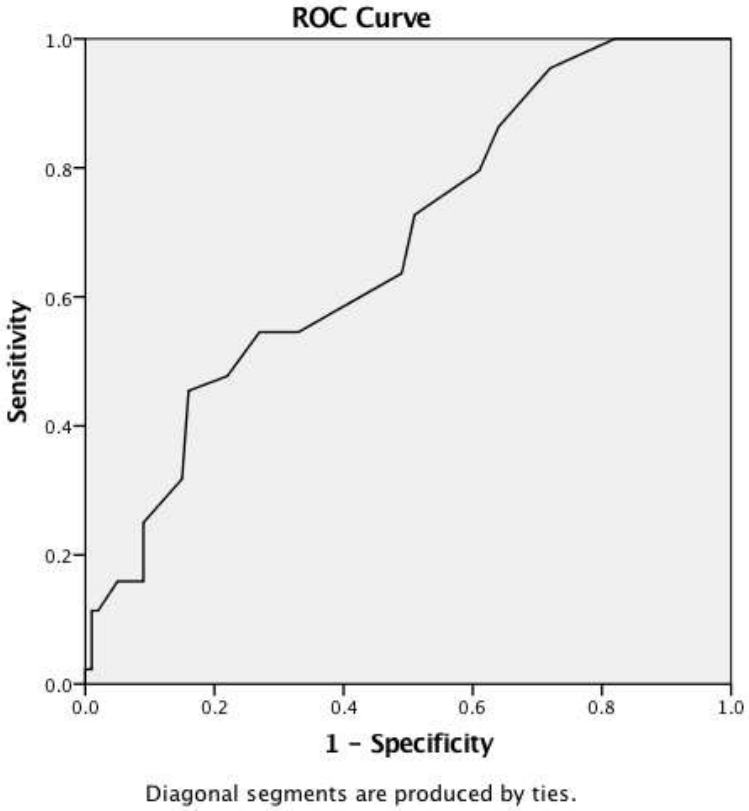

ROC Curve
Sensitivity
1 – Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.

Fig. Vb