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# UNRAVELLING TRANSLATIONAL DYSREGULATION- AN IMMUNOHISTOCHEMICAL EVALUATION OF eIF4E IN SURGICAL MARGINS OF ORAL SQUAMOUS CELL CARCINOMA

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**ABSTRACT** **Objective:** Initiation of eukaryotic translation is a multistep process. It is characterized by the formation of the eIF4F complex, for which the presence of eIF4E is crucial. eIF4E has been extensively evaluated in solid tumors with a 100% overexpression in Oral Squamous Cell Carcinoma (OSCC). The present study aims at assessing the expression of eIF4E in clear and dysplastic surgical margins of OSCC along with a review of literature. **Materials And Methods:** Surgical margins from 30 resected specimens of OSCC were sampled and categorised into three groups - histopathologically clear margins, margins showing low risk oral epithelial dysplasia (OED) and high-risk OED. The expression of eIF4E was immunohistochemically evaluated for each group. **Statistical Analysis:** Statistical analysis was performed using SPSS version 22 and Microsoft Excel for the Chi-square test. **Results:** Surgical margins from all groups demonstrated a variable expression of eIF4E. 46.66% of the clear margins demonstrated a positive expression for the marker. In margins with OED, 71.42% margins with low-risk OED and 75% margins with high -risk OED showed a positivity for eIF4E.A review of literature highlights the need for a standardised approach to evaluate eIF4E in clear and dysplastic surgical margins of OSCC. Since a variable expression has been reported for clear surgical margins while insufficient data exists regarding its expression and activity in dysplastic margins. **Conclusions:** eIF4E overexpression and dysregulation may be seen in histopathogically clear surgical margins. Existing literature has highlighted the fact that there is minimal data regarding the standardised system to categorise sites in oral cavity depending upon the nature of the oral epithelium and expression of various molecular makers. There is a need to have standardised methods and protocols for evaluation of expression of various molecular markers in clear and resected surgical margins with OED. The present study is the first to highlight the expression of eIF4E in dysplastic surgical margins, indicating a probable molecular signature which might aid in predicting their behaviour.

**KEYWORDS :** Oral Squamous Cell Carcinoma, Eukaryotic Initiation Factor-4E, Surgical Margins, Oral Epithelial Dysplasia, Clear surgical margins

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) continues to be the most commonly encountered oral cancer. Surgical management is the primary modality of treatment. Evaluation of surgical margins has been regarded as a key determinant of locoregional control and patient survival (1).

Margin status is reported to be clear (distance >5 mm from invasive tumor cells), close (between 1-5 mm) or involved (<1 mm) based on the standardised and most commonly accepted CAP guidelines (2). Most other systems are equivocal on reporting dysplasia in surgical margins (3).

Local recurrences following the resection of primary tumors have been reported to occur in 10–30% of patients with clear or tumor-free margins (4) and in 66% cases which demonstrate dysplastic margins (5).

This can be attributed to two main pathobiological mechanisms- minimal residual disease (MRD) and the concept of field cancerisation (6).

The presence of isolated histologically undetectable tumor cells within surgical margins, is regarded as MRD (6). These cells pose a substantial challenge for detection during routine margin evaluation. Additionally, there could be the presence of contiguous or distant precancerous fields, comprising of genetically altered cells (6). This phenomenon is regarded as field cancerization. While such areas may not demonstrate any macroscopic changes, histopathological evaluation may reveal innocuous histopathology despite early molecular alterations or even oral epithelial dysplasia, which may be due to the underlying genetic alterations (6). Therefore, histopathologically clear margins may harbour molecular alterations which could potentiate a recurrence.

In an attempt to assess minimal residual disease and field cancerisation at the surgical margins, various techniques have been employed to detect the molecular alterations associated with these mechanisms.

Protein synthesis plays a fundamental role in nearly every aspect of metabolism and constitutes a critical step in the control of gene expression. mRNA translation is divided into three steps; initiation, elongation and termination. Initiation is the rate limiting step of translation and is a subject to extensive regulation.

Eukaryotic initiation factor 4E (eIF4E), is an oncoprotein, regarded as a potential marker of field cancerisation (6). The eukaryotic protein synthesis initiation factor, eIF4E represents the limiting factor in the formation of the eIF4F complex, which is essential for protein translation (7). Interaction with a 4E-binding proteins (4E BPs), exerts an inhibitory effect, preventing formation of eIF4F complex.

Phosphorylation of the eIF4E-BPs by mTOR causes the release of eIF4E and, consequently, a stimulation in translation initiation. Based on their dependence on eIF4E, mRNAs have been classified as “eIF4E-sensitive mRNAs” or “weak mRNAs” and “strong mRNAs”. While strong mRNAs are scarcely influenced by variations in eIF4E, translation of weak mRNAs is strictly dependent on eIF4E. Several weak mRNAs such as cyclins D1 and D3, ornithine decarboxylase (ODC), vascular endothelial growth factor (VEGF), MYC and phosphoribosyl-pyrophosphatase synthetase 2 (PRPS2) code for proteins involved in driving various factors associated with process of carcinogenesis. (7)

eIF4E function is essential for numerous physiological processes, such as protein synthesis, cellular growth and differentiation. Dysregulation and overactivity of eIF4E has been associated with induction of process of carcinogenesis thus making eIF4E a potent oncogene and a central player in the multistep process of carcinogenesis.

eIF4E has been extensively evaluated in solid tumors with a 100% overexpression in tumors of the breast, head and neck and colon (8).

The expression of eIF4E at the surgical margins could aid in identifying early molecular changes in histopathologically clear margins. An updated review of English language literature in relation to eIF4E has highlighted the fact that most of the studies have taken the cumulative heading of head and neck squamous cell carcinoma without any standardized categorization of subsets in oral cavity. Therefore, the present study focuses on evaluation of eIF4E at surgical margins reported histopathogically as clear and margins with grades of OED in resected specimens of OSCC.

## MATERIALS AND METHODS:

Thirty cases of OSCC, treated by surgical resection, were retrieved. Demographic data was recorded and margins were re-assessed using haematoxylin and eosin-stained sections. The margin status was reported as – clear margins, margins showing grades of dysplasia (based on binary grading of OED) (9) and positive margins. Fifteen cases had all surgical margins as clear while fifteen cases had mixed surgical margins which were clear, dysplastic and involved margins.

A single, representative margin from each case was selected grouped into Group A (clear), Group B (low grade OED) and Group B1 (high grade OED) - **(Table: 1)** These groups were assessed for the expression of eIF4E using immunohistochemistry.

**Table 1: Three Study Groups Depending Upon Status Of Surgical Margins**

| **Resection Cases** | Cases with all clear margins | Cases with clear, dysplastic and positive margins | |
|---|---|---|---|
| **Groups** | Group A (n=15) | Group B (n=7) | Group B1 (n=8) |
| **Margin Assessed** | Clear margin | Low grade OED | High grade OED |

n= Number of surgical margins

Formalin fixed paraffin embedded tissue blocks of the selected surgical margins were sectioned at 4 µm and floated onto positively charged hydrophilic slides. The sections were rested and subjected to antigen retrieval using the Heat Induced Epitope Retrieval (HIER). Peroxidase blocking was carried out for ten minutes followed by three rinses with PBS and overnight incubation with the primary antibody (dilution 1:100-Cell Signalling Technologies, USA, eIF4E (C46H6) Rabbit mAb (2067T), (100 ug at 1mg/ml). Slides were rinsed with PBS and incubated with HRP for thirty minutes, followed by DAB substrate and chromogen for ten minutes. Hematoxylin was used as a counter stain and the slides were mounted and viewed. Adenocarcinoma of the colon was used as a positive control and the antibody was omitted for the negative control.

Positive eIF4E expression was defined by the presence of brown perinuclear cytoplasmic staining involving greater that 5% of cells in the basal layer of the epithelium (10). **(Fig 3 d, h i)** Statistical analysis was performed using SPSS version 22 and Microsoft Excel for the Chi-square test. The level of statistical significance was accepted at p value < 0.05.

Literature obtained from databases -Pubmed, Google scholar has shown nominal studies considering evaluation of eIF4E in resected surgical margins. Flow chart in form of PRISMA diagram is made after analysing the obtained articles **(Fig:1)** The relevant information from each article has been reviewed. For the purpose of comparison, the various studies conducted using IHC only (n=10) and IHC with other supplemental techniques(n=5) has been considered and findings have been compared with present study. **(Table:2) (11-25)**

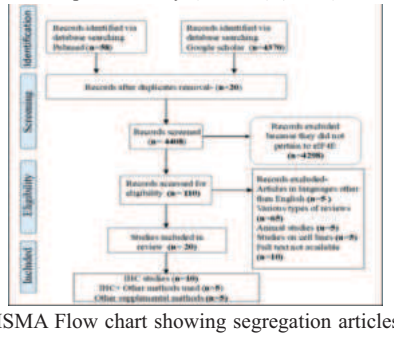



**Fig :1-** PRISMA Flow chart showing segregation articles following review of literature review

## RESULTS:

Thirty resected specimens included cases of Hemi-glossectomy 50 % (15/30), Hemi-mandibulectomy 26% (8/30), Buccal mucosa 13% (4/30), Buccal mucosa along with marginal mandibulectomy 3.3% (1/30), Floor of the mouth along with marginal mandibulectomy 3.3% (1/30) and Composite resection 3.3% (1/30).

A total of 63% (19/30) margins showed a positive expression while 36 % (11/30) were negative for eIF4E. **(Fig. 2)**

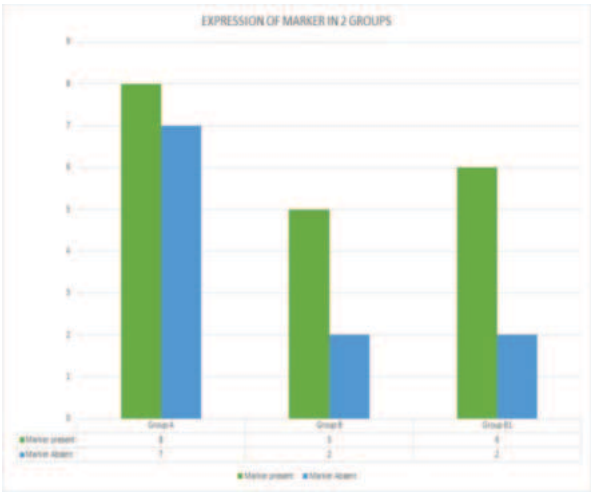
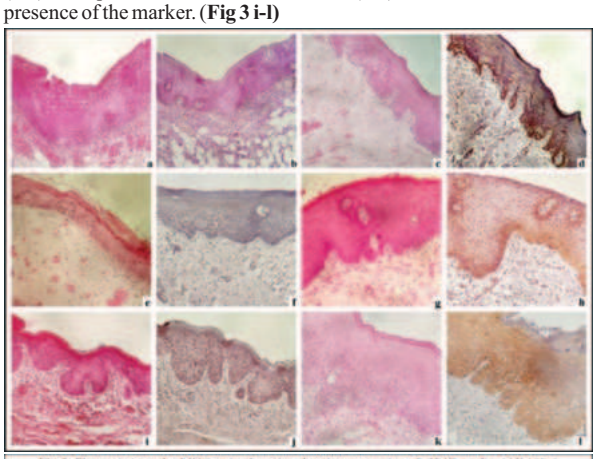



**Fig 2:** Chart presenting as expression of eIF4E in three groups

In group A, comprising of 15 surgically clear margins, 46.66% (7/15) showed positivity for eIF4E while 53.33% (8/15) showed no expression of eIF4E. **(Fig 3 a-d)** No statistically significant difference was noted in the variable expression of eIF4E amongst the clear margins.

Samples from group B, demonstrated low-risk OED, of which 71.42% (5/7) were positive for the expression of eIF4E while 28.57% (2/7) cases were negative for the marker. **(Fig 3 e-h)**

Samples from group B1, demonstrated high-risk OED of which 75% (6/8) were positive for eIF4E while 25% (2/8) cases did not show the presence of the marker. (**Fig 3 i-l)**



Review of literature revealed 15 studies which included an IHC evaluation of eIF4E in the surgical margins of head and neck tumors. On detailed evaluation it has emerged that most studies included tumors from sites beyond the oral cavity with only two stating an evaluation of tumors presenting at various intraoral sites.

No study has exhaustively included tumors from various oral sites and evaluated the status of histologically clear and low and high-risk OED. The present study is the first to report findings exclusive to OSCC presenting in the oral cavity and the variable expression of eIF4E in clear and dysplastic margins. **(Table :2)**

**Table 2: Tabulation Of Findings From Relevant Studies Based On IHC And Other Supplemental Methods Abv. NP-Not provided**

| Author details/ year | Techniques used for evaluation | Aim of the study | Site vise distribution | | | | Conclusion |
|---|---|---|---|---|---|---|---|
| | | | Site /Groups | Margin status | eIF4E +/ eIF4E gene amplification | eIF4E -/Gene amplification not evident | |
| Cherie-Ann O. Nathan et al, 1997 (11) | Western Blot (WB) + IHC | To detect proto-oncogene eIF4E in surgical margins which may predict recurrence in head and neck cancer | Floor of mouth | Clear | 2 | 1 | eIF4E may be a potential prognostic predictor of HNSCC aggressiveness and has a possible role in multistep tumorigenesis process. |
| | | | Base of tongue | Clear | 2 | 0 | |
| | | | Lip | Clear | 1 | 1 | |
| | | | Larynx | Clear | 5 | 6 | |
| | | | Hypopharynx | Clear | 1 | 2 | |
| | | | Tonsil | Clear | 1 | 1 | |
| | | | Total (n=33) | - | 12 | 11 | |
| Cherie-Ann O. Nathan et al, 1999 (12) | IHC | To analysis surgical margins with the molecular marker eIF4E | Oral Cavity (n=11) | Clear | 5 | 6 | Elevated levels of eIF4E in tumor margins may identify patients who could benefit from additional therapy. |
| | | | Maxilla (n=2) | Clear | 0 | 2 | |
| | | | Larynx (n=34) | Clear | 17 | 17 | |
| | | | Hypopharynx (n=6) | Clear | 6 | 0 | |
| | | | Oropharynx (n=12) | Clear | 8 | 4 | |
| | | | Total (n=65) | - | 36 | 29 | |
| Cherie-Ann 0. Nathan, et al, 1999 (13) | IHC | To study the role of 4E in tumorigenesis and further elucidate its causal role in angiogenesis. | Head and neck region | Hyperplasia/ mild atypia (n=41) | 19 | 22 | eIF4E expression is increased during the process of tumorigenesis and could act as one of the factors that causes the switch in angiogenic conversion of premalignant to malignant lesions. |
| | | | | High grade tumor (n=40) | 29 | 11 | |
| | | | | Invasive carcinoma (n=34) | 34 | - | |
| | | | Total (n=115) | - | 82 | 33 | |
| Cherie-Ann O. Nathan et al, 2001 (14) | IHC + WB | To study the correlation of expression of COX-2 with eIF4E as a potential surrogate endpoint for determining response to COX-2 inhibitors. | HNSCC | Normal (n=8) | 0 | 8 | COX-2 inhibitors may play a role in chemoprevention of head and neck cancers and that the correlation of Cox-2 with eIF4E indicates that eIF4E can be a potential surrogate marker in chemoprevention trials. |
| | | | | Hyperplasia with or without mild dysplasia (n=31) | 2 | 29 | |
| | | | | High-grade dysplasia (n=20) | 13 | 7 | |
| | | | | Cancer (n=19) | 19 | 0 | |
| | | | | Margins (n=11) | 5 | 6 | |
| | | | | Total (n=89) | 39 | 50 | |
| Cherie-Ann O. Nathan et al, 2002 (15) | IHC | To analysis eIF4E, p53 and MMP-9 in resected tumor free surgical margins | Oral cavity (n=13) | Clear | 6 | 7 | eIF4E appears to be the most significant predictor of recurrence in HNSCC compared with other well-known factors associated with recurrence. |
| | | | Hypopharynx (n=3) | Clear | 3 | 0 | |
| | | | Larynx (n=25) | Clear | 11 | 14 | |
| | | | Oropharynx (n=11) | Clear | 7 | 4 | |
| | | | Total (n=52) | - | 27 | 25 | |
| Binoy Chandy et al, 2002 (16) | IHC | Expression of elF4E in inflammation | Normal (n=15) | - | 0 | 15 | eIF4E expression was not a significant marker of inflammation. |
| | | | Inflammation (n=29) | - | 4 | 25 | |
| | | | Mild Dysplasia (n=31) | Mild ED | 10 | 21 | |
| | | | Mild dysplasia in surgical margins (n=21) | Mild ED | 9 | 12 | |
| | | | OSCC (n= 15) | Involved/positive | 15 | 0 | |
| | | | Total (n=101) | - | 28 | 73 | |
| Cherie-Ann O. Nathan et al, 2004 (17) | IHC + WB | To determine various markers that upstream and downstream of eIF4E are expressed in eIF4E-positive margins with the intent of targeting the mTOR pathway as adjuvant therapy for patients with eIF4E-positive margins. | Hypopharynx (n=8) | Clear/Involved | NP | NP | Overexpression of eIF4E is functionally active in tumor margins through activation of the Akt/ mTOR signalling pathway. |
| | | | Larynx (n=72) | Clear/Involved | NP | NP | |
| | | | Oral cavity (n=20) | Clear/Involved | NP | NP | |
| | | | Oropharynx (n=11) | Clear/Involved | NP | NP | |
| | | | Total (n=111) | - | NP | NP | |
| Jagtar Singh et al, 2013 (18) | IHC | To determine the prognostic correlation between p53 and eIF4E expression and clinical characteristics, recurrence and overall survival. | Floor of mouth | Clear | NP | NP | Molecular study of surgical margins could help to identify patients with and without clear margins after surgical resection and help to determine the most appropriate adjuvant treatment for HNSCC patients. |
| | | | Tongue | Clear | NP | NP | |
| | | | Lips | Clear | NP | NP | |
| | | | Tonsil | Clear | NP | NP | |
| | | | Total (n=77) | - | NP | NP | |
| Yi Li et al, 2015 (19) | Flow cytometry +IHC | To verify the molecular boundary of OSCC by using p53, p21CIP1/ WAF1 and eIF4E | Tongue (No. of patients =28) | Normal or simple hyperplasia /Mild or moderate dysplasia /Severe dysplasia | T- 50/50<br>P1-36/50<br>P2-24/50<br>P3-19/50 | T- 0/50<br>P1-14/50<br>P2-26/50<br>P3-31/50 | eIF4E overexpression may be an earlier molecular event than p53mutations during OSCC progression. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | expression and to explore the biochemical events in OSCC progression. (Each-n=50) T (centre of the tumor), P1(0–0.5cm to the tumor boundary) P2(0.5-1cm to the tumor boundary) P3(1–1.5cm to the tumor boundary) P4 (1.5–2cmto the tumour boundary) | | /Submucosal invasion of cancer | P4-15/50 | P4-35/50 | |
| | | | Buccal mucosa (No. of patients=22) | | | | |
| | | | Total (Total no. of pts=50) | - | 144/250 | 106/250 | |
| Jagtar Singh et al, 2016 (20) | IHC | To assess prognostic significance of the molecular markers, p53 and eIF4E, in the histologically tumor free surgical margins of HNSCC. | Floor of mouth (n=14) | Clear | 13 | 1 | Expression of eIF4E appears to be a more marked prognosticator compared to p53. |
| | | | Tongue (n=3) | Clear | 2 | 1 | |
| | | | Lips (n=5) | Clear | 4 | 1 | |
| | | | Tonsil (n=2) | Clear | 2 | 0 | |
| | | | Total (n=24) | - | 21 | 3 | |
| Sheela Joseph et al, 2017 (21) | IHC | Study protocol for a prospective, observational and bilateral study in Australia and India for HNSCC-Results unavailable | | | | | |
| Bindhu Joseph et al, 2019 (22) | IHC | To evaluate of elF4E in histologically negative margins | Buccal mucosa (n=15) | Clear | 15 | 0 | eIF4E has a potential to serve as a clinical biomarker of aggressive tumor behavior even prior to morphological changes. |
| | | | Gingivobuccal sulcus (n=8) | Clear | 8 | 0 | |
| | | | Tongue (n=5) | Clear | 5 | 0 | |
| | | | Hard Palate (n=1) | Clear | 1 | 0 | |
| | | | Vocal cord (n=7) | Clear | 7 | 0 | |
| | | | Post cricoid (n=2) | Clear | 2 | 0 | |
| | | | Pyriform sinus (n=3) | Clear | 3 | 0 | |
| | | | Aryepiglottic fold (n=1) | Clear | 1 | 0 | |
| | | | Maxillary sinus (n=1) | Clear | 1 | 0 | |
| | | | Total (n=43) | - | 43 | 0 | |
| Bindhu Joseph et al, 2019 (23) | IHC | To evaluate the potential role of elF4E and p53 as predictive biomarkers in resected margins of oral cancers | Tongue (n=5) | Clear | 1 | 4 | Overexpression of p53 and eIF4E in histologically negative margins of oral cancers may represent a subset of patients with more aggressive tumors who may benefit from early institution of adjuvant radiation. |
| | | | Floor of mouth (n=1) | Clear | 0 | 1 | |
| | | | Gingivobuccal sulcus (n=10) | Clear | 1 | 10 | |
| | | | Retromolar trigone (n=2) | Clear | 0 | 2 | |
| | | | Buccal mucosa (n=21) | Clear | 8 | 13 | |
| | | | Vocal cord (n=10) | Clear | 4 | 6 | |
| | | | Aryepiglottic fold (n=2) | Clear | 1 | 1 | |
| | | | Maxillary sinus (n=1) | Clear | 1 | 0 | |
| | | | Post cricoid (n=3) | Clear | 0 | 3 | |
| | | | Pyriform sinus (n=10) | Clear | 3 | 7 | |
| | | | Total (n=66) | - | 19 | 47 | |
| Chung-I. Huang et al, 2019 (24) | Tissue microarray (TMA) +IHC | To evaluate the association between the expression of proteins in the PI3K/mTOR/eIF4 signalling pathway and the risk of recurrence in patients with HNSCC who have received radical surgery and adjuvant radiotherapy. | 54 cases of HNSCC patients (2 groups -Pts with and without recurrence) | - | All cases showed positive expression of the markers | - | Expression of eIF4E and p-4EBP1 can be considered as predictive biomarkers for the HNSCC patients. |
| Rajul K. Ranka et al, 2021 (25) | IHC | The evaluate the prognostic role of p53, eIF4E and E-cadherin in negative surgical margins and association of their expression with clinical parameters, recurrence, and survival. | Buccal mucosa + gingivobuccal sulcus | Clear | NP | N | Combination of p53, eIF4E and E-cadherin markers expression in histopathologically negative surgical margins could aid to detect minimal residual tumor which might help in better therapeutic management to improve survival of OSCC patients. |
| | | | Tongue | Clear | NP | NP | |
| | | | Lip | Clear | NP | NP | |
| | | | Palate | Clear | NP | NP | |
| | | | Alveolus | Clear | NP | NP | |
| | | | Total (n=64) | - | NP | NP | |
| Present study | IHC | To evaluate the expression of eIF4E in | Hemiglossectomy (n=15) | Clear (n=7) + Margins with ED (n=8) | 8 | 7 | Expression of eIF4E in the resected surgical margins can |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | the resected surgical margins of patients with Oral Squamous Cell Carcinoma. | Hemi-mandibulectomy (n=8) | Clear (n=3) + Margins with ED (n=5) | 4 | 4 | be used to determine prognosis and fortify further treatment protocols. |
| | | | Buccal mucosa (n=4) | Clear (n=2) + Margins with ED (n=2) | 4 | 0 | |
| | | | Buccal mucosal along with marginal mandibulectomy (n=1) | Clear | 1 | 0 | |
| | | | Floor of the mouth along with marginal mandibulectomy (n=1) | Clear | 1 | 0 | |
| | | | Composite Resection (n=1) | Clear | 1 | 0 | |
| | | | Total (n=30) | - | 19 | 11 | |

**Table 3: Tabulation Of Findings From Relevant Studies Based On The Other Supplemental Methods Abv. Np-not Provided**

| Author details/year | Techniques used for evaluation | Aim of the study | Site vise distribution | | | | Conclusion |
|---|---|---|---|---|---|---|---|
| | | | Site /Groups | Margin status | eIF4E +/ eIF4E gene amplification | eIF4E -/ No eIF4E gene amplification | |
| Donald L. Sowells et al, 1999 | Competitive polymerase chain reaction (PCR) +WB | To determine progression in eIF4E gene amplification and protein overexpression in the tumor-free resection margin, the transition zone, and the tumor core of HNSCC 1) tumor core (center of the tumor), 2) transition zone (area at the interface of gross tumor and normal tissue) 3) tumor-free resection margin | 12 HNSCC specimens were divided into three zones and site distribution -Floor of mouth (n=7), Buccal mucosa (n=1), Posterior palate (n=1), Larynx (n=3) | - | All 18 showed gene amplification | - | There is progression of eIF4E gene amplification and overexpression "tumor-free" margin to the tumor core. |
| | | | 10 benign specimens from noncancer patients | - | - | None showed gene amplification-10 | |
| | | | Total (n=22) | - | 18 | 10 | |
| Donald. L. Sorrells et al, 1999 | Competitive PCR +WB | To determine whether eIF4E overexpression is present and associated with eIF4E gene amplification in HNSCC. | 8 HNSCC – Carcinoma of pharyngeal (n=5), oral (n=2), and tonsillar (n=1) | - | All 8 showed gene amplification | - | Overexpression and associated gene amplification of eIF4E were present in HNSCC but not in benign tissue. |
| | | | 7 intraoral benign lesions- tonsils and buccal mucosa from noncancer patients | - | - | None showed gene amplification-7 | |
| | | | Total (n=15) | | 8 | 7 | |
| Donald L. Sorrells et al, 1999 | Quantitative PCR +WB + IHC (IHC of only breast ca -showed + whereas tissue from benign specimens were negative | To determine expression of eIF4E as we progress from - tumor core (center of the tumor) to transition zone (area at the interface of gross tumor and normal tissue), and to the tumor-free resection margin | HNSCC (n=13) | - | Samples of HNSCC and breast carcinoma showed gene amplification (n=23) | - | Overexpression and associated gene amplification of eIF4E were present in HNSCC and IDCB but not in benign tissue. |
| | | | Infiltrating ductal carcinoma of the breast (IDCB) (n=10) | - | | - | |
| | | | Benign specimens from non-cancer patients (n=15) | - | - | None showed gene amplification-15 | |
| | | | Total (n=38) | | 23 | 15 | |
| M. Scott Haydon et al,2000 | Competitive PCR + WB | To evaluate and compare the degree of eIF4E gene amplification and protein overexpression in benign cells of the aerodigestive tract of noncancer patients (normal control), benign tumors, and primary invasive HNCAs. | Benign tissue from noncancer patients (n=10) | - | 0 | Non detectable (n=8) | Progressive eIF4E gene amplification and overexpression were detected with highest degree of amplification in HNSCC followed by benign tumors (pleomorphic adenoma). |
| | | | Benign tumors - pleomorphic adenomas (n=8) | - | Gene amplification - 4 | 4 | |
| | | | Primary invasive HNSCCs (n=18) | - | Detectable levels of gene amplification - 18 | 0 | |
| | | | Total (n=36) | - | 22 | 4 | |
| Gulshan | Semiquantitati | To analyse whether eIF4E | Larynx/oropharynx | - | 8 | 10 | Elevated 4E:4EBP1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sunavala-Dossabhoy et al, 2011 | ve RT-PCR | mRNA could serve as a prognostic maker of HNSCC. | Oral cavity | - | - | 8 | significantly correlated with increased disease recurrence. |
| | | | Total (n=26) | - | 8 | 18 | |

**DISCUSSION:**

A major tenet of surgical management is to achieve local disease control via the physical eradication of tumor cells. The evaluation of surgical margins provides key information regarding residual disease and/or field change. A surgical resection margin is defined as disrupted tissue plane /surface /edge resulting from artificial (surgical) separation from the rest of organs per procedure (26). Histopathologically margins may demonstrate normal mucosa, grades of dysplasia or the infiltration of tumor cells. Routine histopathological evaluation may not suffice in revealing molecular alterations associated with field change, particularly in case of clear surgical margins.

Genetic mutations mainly alter the functions of corresponding proteins, while epigenetic changes will change the expression of potential oncogenes and tumor suppressor genes. The regulation and function of these alterations have been substantially explored. But, the relevance of protein translation or the production of nascent proteins from mRNAs to the initiation and progression in OSCC has been unexplored.

Increased protein synthesis by eIF4E dysregulation modifies the cellular proteome by preferentially upregulating translation of transcripts with long, structured 5′ UTRs, many of which encode proteins linked to cancer or growth promotion.

The prognostic significance of eIF4E has been reported to be superior to p53 when evaluating clear surgical margins (20).

Cherie Ann O. Nathan et al (11,12,15), Bindhu Joseph et al (22,23) and Singh J et al (18,20) reported a variable positivity for eIF4E in clear margins. They concluded that the expression of eIF4E in clear margins indicated molecular alterations in cells which were yet to demonstrate cytological changes. The absence of eIF4E expression was interpreted as a validation for a truly clear margin.

Similar findings were noted in group A of the present study. 63% (19/30) surgical margins showed positivity for eIF4E, highlighting early molecular alterations which were yet to be evident on histopathology. The clear status of 36 % (11/30) margins of group A, was validated by their negative expression of eIF4E.

The presence of OED at surgical margins is a strategic concern. The impact of dysplasia at these sites is viewed ambiguously and renders a prognostic challenge.

A study by Yi Li et al (19) focused on assessing eIF4E in paratumoral mucosa as a guide to establish surgically clear margins. 72% (36/50) cases demonstrated a positive expression of the marker while 28% (14/50) cases were negative for eIF4E. Variable expression within each grade of dysplasia was not detailed as part of their study.

In the present study, a variable expression was noted in the various grades of dysplasia. 28.57% margins with low-risk OED were negative for eIF4E while 25% margins with high-risk OED did not express eIF4E.

The variable expression of eIF4E amongst low and high-risk dysplasia provides an insight into the possible molecular alterations which need not be singularly or sequentially associated with grades of OED. This could also be endorsed by the changing perspective of evaluating dysplasia, which includes differentiating reactive atypia from true OED. (27) Molecular profiling of these entities might aid in an improved recognition of true OED.

There is limited literature on the expression and correlation of eIF4E expression in OED. The definitive interpretation of the variable expression of eIF4E at surgical margins demonstrating OED remains an ongoing challenge and warrants further evaluation.

From the **(Table :2)**, various IHC studies showing the expression of eIF4E in resected surgical margins has highlights the fact that oral cavity is considered as a minority group under the umbrella heading of Head and Neck Squamous cell carcinoma. Due to the complex three-dimensional anatomy of the oral cavity subsites, obtaining adequate surgical margins in cases of OSCC is particularly difficult. This makes accurate interpretation of margins a critical and challenging exercise making oral cavity a unique subset of Head and Neck region. Majority studies in the table following the review of literature have emphasized the fact that all the studies have divided the sites in the oral cavity based upon the location and not based upon the type of resection as recorded in the present study. The status of the resection margin is based upon the site and type of resection advocated to each patient separately.

Prolonged exposure to tobacco associated carcinogens favors the subsets of oral cavity to undergone cellular and molecular changes making it more susceptible for the development of multiple foci of malignant transformation. This necessitates the need for assessment of resected surgical margin not only histopathologically by also using molecular markers. The approach towards the evaluation of surgical margin should be different when there is presence of OED at the resected surgical margins. Thus, the expression of eIF4E in OED needs to be studied further. This was a pilot attempt with site wise evaluation and a standardized approach to evaluation protein translation in OSCC which is required when accounting for the site wise variabilities in the oral cavity.

**CONCLUSIONS:**

The prognosis of OSCC pivots on multiple factors. Tumor stage, tumoral characteristics and status of the surgical margins are key determinants of prognosis.

Translation of selective proteins represent early changes during the process of carcinogenesis. eIF4E acts as an oncogene, preferentially upregulating translation of transcripts with long, structured 5′ UTRs, which drive the process of carcinogenesis.

It has emerged that histopathological evaluation alone, may not suffice in revealing the molecular characteristics of the surgical margins. Therefore, the evaluation of surgical margins using markers such as eIF4E, could aid in detection of high-risk sites in the absence of evident histopathological features. The molecular signature of surgical margins showing OED needs to be evaluated to improve predictability and management strategies. Surgical management of OSCC should aim at achieving margins which are histopathologically clear and molecularly negative to ensure loco-regional control.

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