International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Expression of Epidermal Growth Factor Receptor Protein: A Biological Marker for Oral Pre Cancer and Cancer

Dr. Adithiyan Udayasankar[1], Dr. Pramila Jain[2], Dr. Suryananth KB[3], Dr. Maneesh Sulya[4], Dr. Sonal Gupta[5]

[1]Postgraduate Resident, Department of Pathology, Gandhi Medical College, Bhopal

[2]Professor, Department of Pathology, Gandhi Medical College, Bhopal

[3]Postgraduate Resident, Department of Community Medicine, Gandhi Medical College, Bhopal
Corresponding Author Email: *suryananthkb[at]gmail.com*

[4]Professor & Head, Department of Pathology, Gandhi Medical College, Bhopal

[5]Associate Professor, Department of Pathology, Gandhi Medical College, Bhopal

Abstract: ***Background: OSCCs aetiology is complex with numerous intrinsic and extrinsic causes, account for over 94% of oral malignancies in India. Survival, metastasis, and tumour growth have all been linked to high EGFR expression. Overexpression of EGFR, most likely as a result of increased intrinsic proliferative activity. Objective: Study the expression of EGFR in Premalignant and Malignant Oral Lesion. Methodology: Cross - sectional study conducted in dept. of pathology for 1.5 years using a questionnaire containing information related to risk factors of oral pre malignant and malignant condition after sectioning the tissues. Inclusion criteria: All tissues of oral biopsy which were histologically reported as proven case of Oral premalignant lesions and Oral Squamous Cell Carcinoma collected from 1st August 2022 to 31st January 2024 in the department of pathology, Gandhi Medical College, Bhopal. Exclusion criteria: Biopsies with tissue insufficient for histopathological evaluation, Recurrent cases, and patients taken neo - adjuvant therapy, Autolyzed samples. Result: 103 patients, mean age was 52.1±14.5years. Tobacco chewing reported by 92 (89.3%). The most common biopsy site was the buccal mucosa (58.3%). Chi - square test shows a statistically significant association between age category and lesion type ($\chi^2 = 12.6$, $p = 0.028$). Dysplasia (mild and moderate) exhibited 31.5% high EGFR expression, 31.5% low expression, and 37% with no expression. Conclusion: Increased EGFR expression correlates with the progression of oral lesions from premalignant to malignant stages.***

Keywords: OSCC, EGFR, Oral cancer, Pre - malignant, Malignant

1. Introduction

Oral cancer is the sixth most prevalent type of cancer worldwide, with India accounting for about one - third of cases and ranking second in terms of the number of instances. [1] The most important factor influencing a successful course of treatment, improved prognosis, and survival from cancer is early detection, which goes beyond prevention. [2] Oral squamous cell carcinomas (OSCCs), whose aetiology is complex with numerous intrinsic and extrinsic causes, account for over 94% of oral malignancies in India. [3] Tumorigenesis is significantly influenced by inflammation, which is also a result of bacterial and viral infections and inflammatory bowel disorders, both of which increase the risk of cancer. The histopathology demonstrates how oral cavity carcinomas progress from basic dysplasia to highly invasive tumours. [4] Growth factors and their receptors on the surface of cancer cells often regulate the growth and differentiation of cancer in the body. [5] Many cancer cell types have abnormally high quantities of it on their surface, which might cause the cells to divide uncontrollably when epidermal growth factor is present. Survival, metastasis, and tumour growth have all been linked to high EGFR expression. Compared to tumours with low - level EGFR expression, oral tumours overexpressing EGFR show a higher percentage of full responses to targeted chemotherapy. Overexpression of EGFR, most likely as a result of increased intrinsic proliferative activity, may make drugs more hazardous to cells going through mitosis and increase susceptibility to treatment. [6]

Objective: To study the expression of EGFR in Premalignant and Malignant Oral Lesion.

2. Material & Methods

Study centre: Department of Pathology, Gandhi Medical College, Bhopal

Study duration: 1st August 2022 to 31st January 2024.

Study Design: Cross - sectional study.

Data collection:

1) Samples were received as formalin fixed tissue samples of oral biopsy received in Department of Pathology, Gandhi Medical College and Hamidia Hospital, Bhopal.
2) Information or history was taken from requisition forms received in Department of Pathology.

Inclusion criteria:

1) All tissues of oral biopsy which were histologically reported as proven case of Oral premalignant lesions and Oral Squamous Cell Carcinoma collected from 1st

August 2022 to 31st January 2024 in the department of pathology, Gandhi Medical College, Bhopal.

Exclusion criteria:

1) Biopsies with tissue insufficient for histopathological evaluation.
2) Recurrent cases, and patients taken neo - adjuvant therapy.
3) Autolyzed samples.

3. Methodology

After grossing, the tissues were processed and paraffin blocks were made and cut using microtome. The slides were prepared, processed and stained with hematoxylin and eosin. The samples were processed for histopathology evaluation and immunohistochemistry.

Histopathology Staining: [7]

- Tissue sections were taken from the specimen received.
- These sections were fixed in 10% formalin overnight at room temperature processed and embedded in paraffin wax.
- Four micrometer sections were cut, de - paraffinized and stained with H&E stains.

Hematoxylin and Eosin Staining Procedure: [8]

- Sections were de - waxed in two jars of xylene for two minutes each.
- Xylene removed by keeping slides in two jars of absolute alcohol, each for two minutes.
- Treatment with descending grades of alcohol, in 90% alcohol for 2 minutes & 70% alcohol for 2 minutes then rinse in tap water.
- Sections were kept in harris - hematoxylin for 7 - 10 minutes.
- Washing under running tap water till the sections turn blue.
- Sections differentiated in 1% acid alcohol for 5 - 10 seconds.
- Washing in tap water for 5 minutes. Dipped in saturated solution of lithium carbonate till the section is completely blue. Washing in tap water for 10 minutes.
- Treatment with increasing grades of alcohol, in 50% alcohol for 2 minutes, 70% alcohol for 2 minutes & 90% alcohol for 2 minutes.
- Counter stain with 1% Eosin Y for 1 minute.
- Rinse in 95% alcohol two times each for 2 minutes.
- Dehydrate with absolute alcohol three times each for 2 minutes.
- Clearing done by three changes in xylene each for two minutes. Mount in DPX.

Result of H&E Staining:

- Cell nuclei - Stain blue Cytoplasm - stain eosinophilic red blood cell - stain orange/ Red
- The slides were Histologically reported as Oral premalignant lesions and Squamous Cell Carcinoma by faculty in Department of Pathology, Gandhi Medical College, Bhopal.
- After HPE, IHC staining was done for marker EGFR.

Immunohistochemistry Steps:

- Cases reported histo - pathologically were then subjected to immunohistochemistry.
- Tissue mounted on poly lysine coated slides was stained for EGFR.

IHC Procedure: [9]

- Prior to cutting sections, paraffin blocks were placed on ice blocks for a few minutes.
- 3 - 4 micrometer thick sections were cut and gently lowered on surface of water bath at 450c and was spread wrinkle free on the poly L - lysine coated slide.
- Baking: Slides were further reheated on plate at 60 °c for 30 minutes.
- De warming and rehydration: Slides were further dipped in coplins jar containing
- Xylene - I for 10 minutes, Xylene - II for 10 minutes, Absolute alcohol for 5 minutes, 90% alcohol for 5 minutes, 70% alcohol for 5 minutes.
- Slides were placed in a pressure cooker with preheated citrate buffer and cooked for one whistle before being allowed to cool naturally for 20 minutes.
- Slides were then washed with PBS for 3 times for 3 minutes each.
- Blocking endogenous enzyme: Slides were wiped thoroughly with tissue paper and incubated in Ultra Vision Hydrogen Peroxide Block for 10 minutes in moist chamber at room temperature in order to reduce nonspecific background staining due to endogenous peroxidase followed by buffer wash step.
- Ultra vision Protein Block was applied and incubated for 5 minutes to block nonspecific background staining followed by buffer wash step.
- Primary antibody (EGFR) was added and incubated in moist chamber for 60 minutes. Buffer wash step.
- Amplification of Primary Antibody: Primary antibody Amplifier Quanto was added and incubated in moist chamber for 20 minutes followed by buffer wash step.
- HRP polymer Quanto was used as a secondary antibody, and it was incubated in a moist chamber for 20 minutes followed by buffer wash step.
- Substrate: 30 micro liter/ 1drop DAB QUANTO CHROMOGEN was added lo 1ml of DAB Quanto Substrate, mixed by swirling movement was applied to tissue and incubated for 5 minutes. Distilled water wash step.
- Slides were then counterstained with Hematoxylin and cover slips were applied onto them using DPX.

Positive Controls

Positive control section included mucoepidermoid carcinoma of salivary gland and was treated in the same manner as the test groups.

Negative Controls

One section of normal skin was selected and treated in the same manner as the test groups except that, the primary antibody was omitted for EGFR Immuno - histochemistry slides were evaluated by two pathologists unbiased from each other.

Assessment of EGFR: [10]

Pathologists with experience assessed immunostaining. The presence of a particular stain on the surface membrane of

tumour cells was classified as antigen expression for EGFR. The product of a proportion score and an intensity score was used to compute a total immunostaining score.

Statistical Analysis:
Data thus collected was entered in MS Excel and analysed using Epi info 7.2.1 software. Descriptive statistics like mean, standard deviation, median, interquartile range were calculated for the quantitative data. Frequency and percentage were calculated for the qualitative data. Inferential statistics like Chi - square and Fischer exact P test were applied between two categorical data. Normality test was done. For data with normal distribution, Independent T test was applied between qualitative data and quantitative data. For data with non - normal distribution, Mann Whitney U test was applied between qualitative data and quantitative data. The P - value <0.05 was considered statistically significant.

Ethical Consideration
Our study was approved by the Institutional Ethics Committee of Gandhi Medical College and Hamidia Hospital, Bhopal (M. P.), having IEC no.50/IEC/2022.

4. Result

The study comprised 103 patients, with 24 females (23.3%) and 79 males (76.7%). The study included patients with a wide age range from 16 to 90 years. The mean age of the patients was 52.1±14.5years. The majority of patients were between the ages of 51 to 60 years (25%), representing 24.3% of the sample. The youngest age group <30 years accounted for 5.8%, and the oldest age group >70 years represented (10.7%) of the patients. The study patients reported various risk factors associated with oral lesions. The most common risk factor was tobacco chewing reported by 92 (89.3%) of patients. Sharp teeth or dentures were cited by 79 (76.7%) of patients, indicating a significant prevalence of mechanical irritation factors. Alcohol consumption was noted by 60 (58.3%) of patients, while cigarette smoking was reported by 57 (55.3%). The most common duration of smoking was 10 to 20 years (23.3%), tobacco chewing tobacco was 20 to 30 years (43.7%) and alcohol consumption was 10 to 20 years (22.3%). (55.3%) had tobacco - stained teeth (6 to 10). Majority (89.3%) reported poor oral hygiene. The most common biopsy site was the buccal mucosa (58.3%). The majority of the biopsy were taken from the right side (53.4%). (48.5%) were diagnosed less than 3 months of duration. (68.0%) had malignant lesions, while 32.0% were diagnosed with premalignant lesions. Premalignant lesions were most prevalent among individuals aged 41 to 70 years, with peaks in the 41 to 50 age group (27.3%) and the 61 to 70 age group (27.3%). Malignant lesions showed higher percentages in 30 to 60 age groups, particularly predominant in 30 to 40 age group and 51 to 60 age group (24.3%). The chi - square test shows a statistically significant association between age category and lesion type ($\chi^2 = 12.6$, $p = 0.028$). Mild dysplasia is most prevalent in the 61 - 70 age group with 5 cases (45.5%). Moderate dysplasia is found in the 41 - 50 and 51 - 60 age groups with 2 cases each (33.3%), and single cases in the 30 - 40 (16.7%) and >70 (16.7%) age groups. Among malignant lesions, 60.0% were cigarette smokers. Among premalignant lesions, 81.8% were tobacco users. Among premalignant lesions, 63.6% reported alcohol use, malignant lesions, 55.7% were alcohol users. Malignant lesions, 80.0% used dentures.

Table 1: Association of EGFR expression in various premalignant lesions

	EGFR Expression		
Histological diagnosis	High	Low	No expression
Keratosis (Leucoplakia)	3 (50.0 %)	1 (16.7 %)	2 (33.3 %)
Oral keratosis with mild dysplasia	1 (100.0 %)	0 (0.0 %)	0 (0.0 %)
Dysplasia (mild & moderate)	6 (31.5 %)	6 (31.5 %)	7 (37 %)
Carcinoma in situ (severe dysplasia)	3 (60 %)	2 (40 %)	0 (0.0 %)

Test	χ^2 Value	df	p
Fisher's exact test	14	6	0.419

Based on the analysis of EGFR expression in various premalignant oral lesions, there are notable differences in the levels of EGFR expression across different histological diagnoses. Oral keratosis with mild dysplasia showed 100% high EGFR expression with no cases of low or no expression. Carcinoma in situ (severe dysplasia) had high EGFR expression in 60% of cases and low expression in 40%, with no cases showing no expression. Dysplasia (mild and moderate) exhibited 31.5% high EGFR expression, 31.5% low expression, and 37% with no expression. Keratosis (Leucoplakia) showed 50% high expression, 16.7% low expression, and 33.3% with no expression. Statistical analysis using Fisher's exact test did not reveal a significant difference in EGFR expression among these premalignant lesions ($p = 0.419$), indicating that the variability in EGFR expression is not statistically significant across different types of premalignant oral lesions.

Table 2: Association of EGFR expression in various malignant lesions

	EGFR Expression		
Histological diagnosis	High (TS>=4)	Low (TS<4)	No expression (TS=0)
IWDSCC	40 (69 %)	14 (24 %)	4 (7%)
IMDSCC	8 (88.8 %)	1 (11.2 %)	0 (0.0 %)
IPDSCC	1 (100.0 %)	0 (0.0 %)	0 (0.0 %)

Test	χ^2 Value	df	p
Fisher's exact test	1.46	4	0.727

The table outlines the distribution of EGFR expression levels across various histological diagnoses of malignant lesions, revealing no statistically significant association ($p = 0.727$). Invasive moderately differentiated squamous cell carcinoma (IMDSCC) exhibited high EGFR expression in 88.8% of cases, low expression in 11.2%, and no cases with no expression. Invasive poorly differentiated squamous cell carcinoma (IPDSCC) had 100% high EGFR expression, with no cases showing low or no expression. Invasive well - differentiated squamous cell carcinoma (IWDSCC) had high expression in 69% of cases, low expression in 24%, and no expression in 7%. Other malignant lesions showed high EGFR expression in 50% of cases, low expression in 25%, and no expression in 25%. These findings underscore the heterogeneous nature of EGFR expression among different types of malignant lesions. Out of total 103 patients, 58

patients were diagnosed with IWDSCC, out of which 4 patients had total score of 0, 6 patients had a total score of 1, 4 patients had a score of 2, 5 patients had a score of 3, 2 patients had a score of 4, 13 patients had a score of 6, 1 patient had a score of 8, 15 patients had a score of 9, 8 patients had a score of 12.11 patients were diagnosed with IMDSCC, 1 patient had a score of 0, 1 patient had a score of 1, 1 patient had a score of 6, 4 patients had a score of 9, 4 patients had a score of 12.1 patient was diagnosed with IPDSCC who had a score of 9.

Table 3: Association of premalignant and malignant lesions with EGFR expression

	EGFR Expression			
Lesion Type	High	Low	No expression	Total
Pre malignant	15 (45.5 %)	9 (27.3 %)	9 (27.3 %)	33 (100.0 %)
Malignant	49 (70.0 %)	16 (22.9 %)	5 (7.1 %)	70 (100.0 %)
Total	64 (62.1 %)	25 (24.3 %)	14 (13.6 %)	103 (100.0 %)

Test	Value	df	p
χ^2	9.04	2	0.011

The above table examines the relationship between EGFR expression levels and premalignant and malignant lesions, indicating a significant association ($\chi^2 = 9.04$, $p = 0.011$). Notably, a larger proportion of malignant lesions (70.0%) exhibited high EGFR expression compared to premalignant lesions (45.5%), where 27.3% showed low EGFR expression and another 27.3% had no expression. In contrast, among malignant lesions, 22.9% showing low expression and 7.1% demonstrating no expression. This suggests a potential role of EGFR expression in distinguishing between premalignant and malignant states in the studied cases.

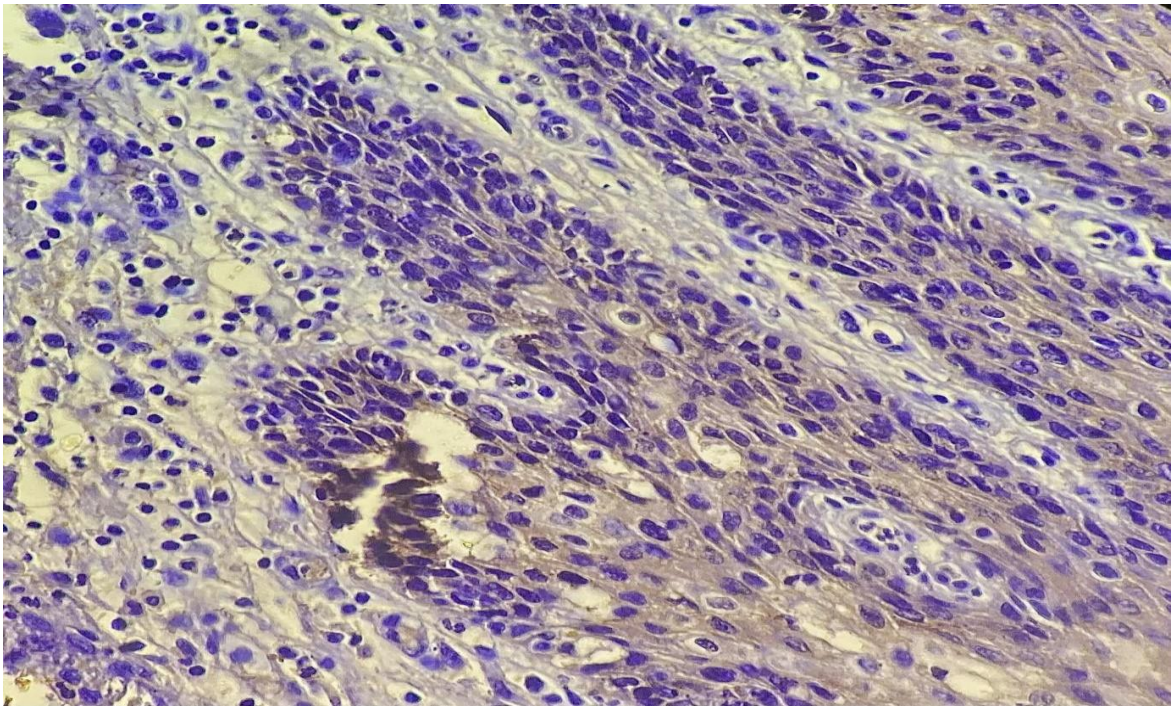

Figure 1: Shows Low EGFR expression in a case of Mild Dysplasia (400X)

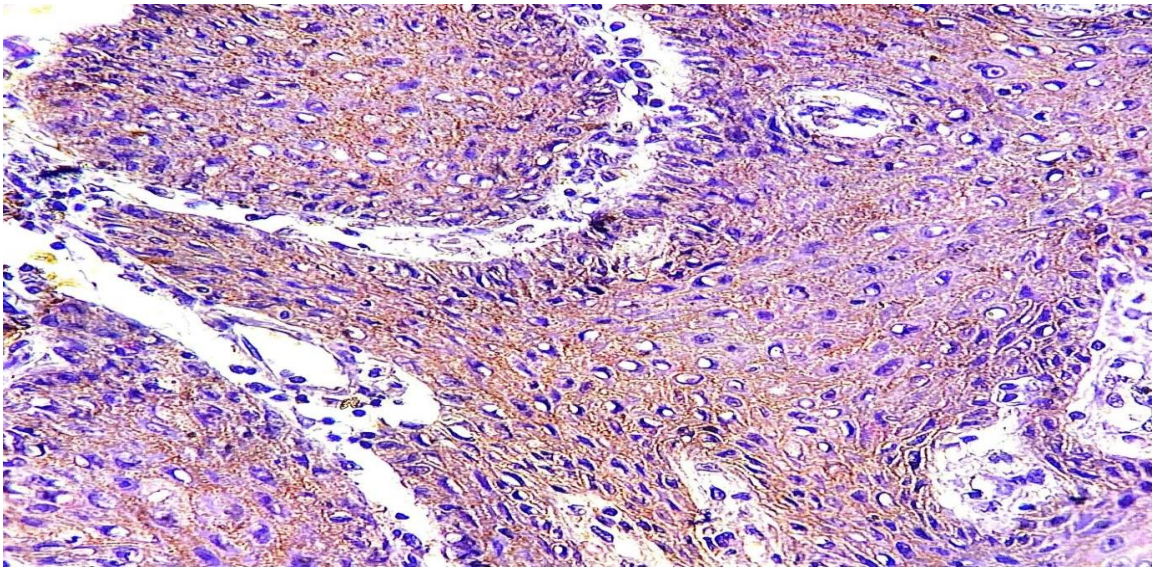

Figure 2: Shows high EGFR expression in a case of Severe Dysplasia (400X)

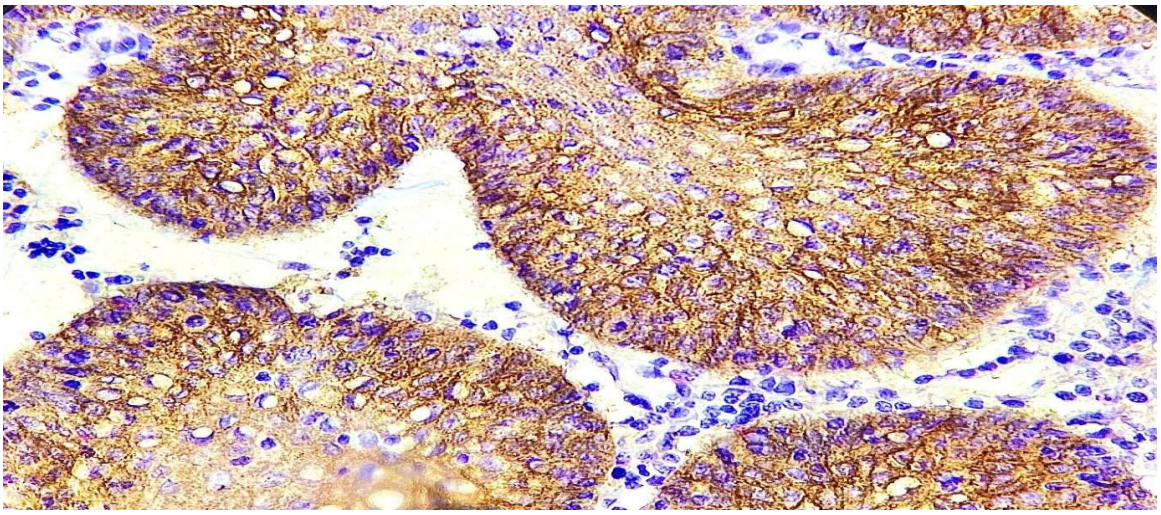

Figure 3: Shows high EGFR expression in a case of infiltrating well differentiated squamous cell carcinoma (400X)

5. Discussion

The mean age of patients in our study was 52.1 ± 14.5 years, which is consistent with the study by Noor M et al. [11] (52.2 ± 13.1 years). The gender distribution in our study shows a higher prevalence of male patients (77%) compared to female patients (23%). This is different from other studies, such as Zhou X et al. [12] (64.3% male, 35.7% female). Our study shows malignant lesions consistently exhibit higher EGFR scores compared to premalignant lesions, indicating potentially more extensive EGFR expression. Similar results were there in the study conducted by Cortés - Ramírez DA et al. [13] Malignant lesions consistently exhibited higher EGFR expression levels compared to premalignant lesions, with 70.0% of malignant cases showing high EGFR expression compared to 45.5% in premalignant lesions. This is similar to the study conducted by Mahendra A, Shreedhar B et al. [14] and Mirza et al. [15] Our study further analyzed EGFR expression in different types of malignant lesions: IMDSCC, IPDSCC, and IWDSCC. High EGFR expression was observed in 69% of IWDSCC, 88.8% of IMDSCC and 100% of IPDSCC. Low EGFR expression was seen in 24% of IWDSCC, 11.2% of IMDSCC, 0% of IPDSCC. Comparatively, the study by Santiago Chile et al. [16] reported high EGFR expression in 73% of OSCC and low EGFR expression in 27% of OSCC.

6. Limitations

- While our findings support EGFR as a promising biomarker for distinguishing between different stages of oral lesions and guiding therapeutic decisions, the study's retrospective design and limited sample size represent notable limitations.
- We were not able to follow up the patients due to attrition bias. So, the final outcome of the lesion could not be assessed for ascertaining the prognosis.

7. Conclusion

EGFR (Epidermal Growth Factor Receptor) expression has been recognized as a significant biological marker in oral premalignant and malignant lesions. Our study showed that increased EGFR expression correlates with the progression of oral lesions from premalignant to malignant stages. EGFR biomarker has implications for early detection, prognosis, and targeted therapy in oral cancer. EGFR expression serves as a valuable indicator in identifying and monitoring the development of oral premalignant lesions towards

malignancy. Its role in clinical practice highlights its potential for improving diagnostic accuracy and guiding personalized treatment strategies, ultimately contributing to better outcomes for patients with oral cancer.

References

[1] Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sens Int.2020; 1: 100046.
[2] Weigum SE, Floriano PN, Christodoulides N, McDevitt JT. Cell - based sensor for analysis of EGFR biomarker expression in oral cancer. Lab Chip.2007; 7: 995–1003.
[3] Tandle AT, Sanghvi V, Saranath D. Determination of p53 genotypes in oral cancer patients from India. Br J Cancer.2001; 84: 739–42.
[4] Dascălu I. Histopathological Aspects in Oral Squamous Cell Carcinoma. Open Access Journal of Dental Sciences.2018; 3.
[5] Molecular Biology of Human Oral Cancer - D. T. W. Wong, R. Todd, T. Tsuji, R. B. Donoff, 1996 [Internet]. [cited 2025 Feb 28]. Available from: https: //journals. sagepub. com/doi/abs/10.1177/10454411960070040201
[6] Hiraishi Y, Wada T, Nakatani K, Negoro K, Fujita S. Immunohistochemical expression of EGFR and p - EGFR in oral squamous cell carcinomas. Pathol Oncol Res.2006; 12: 87–91.
[7] Stambuk HE, Karimi S, Lee N, Patel SG. Oral cavity and oropharynx tumors. Radiol Clin North Am.2007; 45: 1–20.
[8] [PDF] A Review of Biopsy in Dentistry: Principles, Techniques, and Considerations | Semantic Scholar [Internet]. [cited 2025 Feb 28]. Available from: https: //www.semanticscholar. org/paper/A - Review - of - Biopsy - in - Dentistry%3A - Principles%2C - and - Zargaran/d30174c5295a5d3fd8c7c763b833f3f4096dabfb
[9] Mehrotra R, Mishra S, Singh M, Singh M. The efficacy of oral brush biopsy with computer - assisted analysis in identifying precancerous and cancerous lesions. Head Neck Oncol.2011; 3: 39.
[10] Sajjanar M, Sajjan P, Puranik RS, Vanaki S, Jayanth V, Naik P. “Analysis of immunohistochemical expression of epidermal growth factor receptor in oral squamous cell carcinoma using tissue microarray technology and whole sections: ” A comparative study. J Oral Maxillofac Pathol.2020; 24: 499–504.
[11] Noor M, Ishaq Y, aftab A, Ata S, Manji S, Anwar MA. Diagnostic Accuracy of Lugol’s Iodine Staining in Detection of Safe Margins of Oral Squamous Cell Carcinoma. Health Science Journal.2020; 14: 725.
[12] Li X, Rong H, Zhang J, Wang D, Li Y. Modulating the local coordination environment of single - atom catalysts for enhanced catalytic performance. Nano Res.2020; 13: 1842–55.
[13] Keshani F, Kargahi N, Nikbakht MH, Najafi S, Fallah F. Expression of epithelial growth factor receptor as a protein marker in oral reticular and erosive lichen planus. BMC Oral Health.2024; 24: 726.
[14] Cortes - Ramirez Da, Rodriguez - Tojo Mj, Coca - Meneses Jc, Marichalar - Mendia X, Aguirre - Urizar Jm. Epidermal growth factor receptor expression in different subtypes of oral lichenoid disease. Med Oral.2014; e451–8.
[15] Mirza Y, Ali SA, Awan MS, Idress R, Naeem S, Zahid N, et al. Overexpression of EGFR in oral premalignant lesions and OSCC and its impact on survival and recurrence. Department of Surgery [Internet].2018 [cited 2025 Mar 1]; Available from: https: //scholars. aku. edu/en/publications/overexpression - of - egfr - in - oral - premalignant - lesions - and - oscc - and -
[16] Adorno - Farias D, Badilla SM, Vidal GP, Fernandez - Ramires R. Epidermal Growth Factor Receptor (EGFR) is Overexpressed in Both Dysplasia and Neoplastic Oral Diseases.2022; 13.