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Evaluation of Human Epidermal Growth Factor Receptor-2 (HER2/neu) Expression in Oral Squamous Cell Carcinoma: An Immunohistochemical Study

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Abstract

Background

Overexpression of human epidermal growth factor receptor-2 (HER2/neu) has been well-established in some epithelial malignancies, but its function in oral squamous cell carcinoma (OSCC) is still debatable and varies greatly between studies. Finding trustworthy prognostic and therapeutic biomarkers is still difficult. The aim of this study is to evaluate the immunohistochemical expression of HER2/neu in oral squamous cell carcinoma and assess its potential relevance as a biomarker in an Indian population.

Materials and methods

This cross-sectional observational study included 43 histopathologically confirmed cases of OSCC. Formalin-fixed, paraffin-embedded tissue sections were subjected to immunohistochemistry for HER2/neu using a standardized protocol. Appropriate positive and negative controls were employed. HER2/neu expression was assessed based on membranous staining patterns and interpreted according to established scoring criteria.

Results

All 43 OSCC cases demonstrated the absence of HER2/neu expression, with no membranous staining observed in tumor cells. Internal and external controls showed appropriate staining, confirming the technical adequacy of the procedure. No association could be established between HER2/neu expression and clinicopathological parameters due to uniform negativity.

Conclusion

HER2/neu membranous expression was absent in all 43 OSCC cases included in this study. These findings indicate that HER2/neu overexpression was not detected in the study cohort. However, the results should be interpreted cautiously in view of the limited sample size and single-center design. Larger multicentre studies incorporating molecular validation, including HER2 gene amplification analysis, are required to further clarify the potential significance of HER2/neu in OSCC.

Categories: Other, Pathology, Oncology
Keywords: biomarker, her2/neu, immunohistochemistry, negative expression, oral cavity squamous cell carcinoma

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy that occurs in the oral region and is a major public health problem, especially in South Asian countries where tobacco chewing, smoking, and alcohol consumption are more common, including risk factors [1]. Despite progress in surgical approaches and adjuvant treatment, survival for OSCC has had modest improvement overall due to late diagnosis presentation, tumor recurrence, and biological diversity [2].

Traditional forecasting tools in oral cancer are highly dependent on histopathological factors like Tumor Node Metastasis (TNM) stage and tumor grade. However, such parameters frequently inadequately represent the underlying biology of a tumor and fail to consistently predict response to therapy or patient outcome [3]. This limitation has led to emerging interest in molecular and immunohistochemical markers that might aid routine histopathological diagnosis and risk stratification of OSCC [4].

The human epidermal growth factor receptor-2 (HER2/neu) is a transmembrane tyrosine kinase receptor in cell proliferation and survival signaling pathways. HER2/neu overexpression and gene amplification are

well-established prognostic and predictive markers in breast32,33 and gastric carcinomas34-36 where targeted therapy has dramatically changed clinical outcome [5]. HER2/neu has been investigated as a biomarker in OSCC, considering its implication in epithelial neoplasia.

However, the reports on HER2/neu expression in OSCC are conflicting and differential in nature, with positivity rates varying from low to high. These inconsistencies are thought to be a result of variation in study population, site of tumor, technical methods, antibody selection, and scoring system [6]. In addition, the absence of standardized interpreting algorithms for HER2/neu in OSCC would further interfere with comparison between studies.

Recent literature indicates the absence of an individual biomarker with consistent diagnostic or prognostic value in OSCC, and continued screening of molecular markers along with clinicopathological characteristics is necessary [7]. This emphasizes the need for population-specific studies to evaluate patterns of biomarker expression and the associated potential impact on patient management.

In this background, the present work was conducted to analyze the immunohistochemical expression of HER2/neu in oral squamous cell carcinoma and its association as a biomarker in our population.

Materials And Methods

Study design

This cross-sectional observational study was carried out in the Department of Pathology at the All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha, India, a tertiary care teaching hospital. The study was performed on formalin-fixed, paraffin-embedded tissue samples of oral squamous cell carcinoma.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS) Bhubaneswar (approval no. AIIMS BBSR/PG Thesis/2022-2023/46). The study utilized archived formalin-fixed, paraffin-embedded human tissue specimens obtained from the departmental archives. The requirement for individual informed consent was waived by the Institutional Ethics Committee, subject to its approval and applicable institutional guidelines.

Study material

A total of 43 histopathologically confirmed cases of oral squamous cell carcinoma were included in the study. Cases were retrospectively identified from the departmental archives during the study period from January 2023 to December 2025. All eligible cases available during this period were included consecutively. Histopathological confirmation of OSCC was based on the corresponding hematoxylin and eosin-stained sections, and formalin-fixed, paraffin-embedded tissue blocks with adequate tumor tissue were selected for further analysis.

Inclusion and exclusion criteria

Histopathologically confirmed cases of oral squamous cell carcinoma and adequate formalin-fixed, paraffin-embedded tissue available for immunohistochemical analysis were included. Inadequate or poorly preserved tissue samples; cases with extensive necrosis or insufficient tumor tissue; and patients who had received neoadjuvant chemotherapy or radiotherapy prior to tissue sampling were excluded.

Histopathological evaluation

Hematoxylin and eosin-stained sections from each case were reviewed to confirm the diagnosis and tumor type. The tumors were classified and graded according to the histopathological criteria outlined in the World Health Organization (WHO) Classification of Head and Neck Tumours.

Immunohistochemistry for HER2/neu

Immunohistochemistry was performed on 4-µm-thick paraffin-embedded tissue sections. Heat-induced epitope retrieval was conducted using a microwave oven for 10 minutes at 100°C in Tris-EDTA buffer. Immunohistochemical staining was performed using a rabbit monoclonal anti-HER2/ErbB2 antibody (clone EP3; PathnSitu Biotechnologies, India; catalog no. HAR047-6ML). The antibody was supplied in a ready-to-use formulation and was used according to the manufacturer's instructions. The manufacturer identifies EP3 as a rabbit monoclonal antibody, with membranous localization and breast carcinoma as the recommended positive control.

Controls

Known HER2/neu-positive breast carcinoma tissue was used as a positive control. Sections processed without the primary antibody served as negative controls. All controls showed appropriate staining,

confirming technical adequacy.

Interpretation of staining

HER2/neu expression was evaluated based on membranous staining of tumor cells. Cytoplasmic staining, when present, was considered nonspecific and excluded from interpretation. Immunohistochemical staining was assessed using a four-tier scoring system (0, 1+, 2+, and 3+) based on staining intensity, completeness of membrane staining, and the proportion of tumor cells showing membranous reactivity.

Score 0 was assigned when no membranous staining was observed or when incomplete, faint membranous staining was present in ⩽10% of tumor cells. Score 1+ indicated incomplete, faint or barely perceptible membranous staining in more than 10% of tumor cells. Score 2+ represented weak to moderate but complete circumferential membranous staining in more than 10% of tumor cells. Score 3+ indicated strong, complete circumferential membranous staining in more than 10% of tumor cells.

For the purpose of this study, only score 3+ staining was considered positive for HER2/neu expression. This stringent threshold was adopted to identify unequivocal membranous overexpression and minimize false-positive interpretation associated with weak or incomplete staining. Scores 0, 1+, and 2+ were considered negative for the primary study outcome, with score 2+ representing equivocal staining. Fluorescence in situ hybridization (FISH) was not performed for further evaluation of equivocal cases.

HER2/neu immunohistochemical staining was independently evaluated by two pathologists using a blinded assessment approach. Both observers were blinded to the clinical and histopathological details of the cases and independently assessed the intensity, completeness, and extent of membranous staining in tumor cells using the predefined four-tier scoring system (0, 1+, 2+, and 3+). The scores assigned by the two observers were recorded independently, and interobserver agreement was assessed to evaluate the reproducibility of HER2/neu immunohistochemical scoring.

Statistical analysis

Data were recorded and analyzed descriptively. Due to the uniform absence of HER2/neu expression in all cases, statistical correlation with clinicopathological parameters was not performed.

Results

A total of 43 histopathologically confirmed cases of oral squamous cell carcinoma were included in the study. Adequate tumor tissue was available in all cases for immunohistochemical evaluation. Clinicopathological characteristics of the study population are presented in Table *1*. Clinical staging information was unavailable for 11 cases due to incomplete documentation in the archived medical records.

Characteristic	Category	n	%
Total patients	Overall	43	100
Age (years)	Range	27–77	
Sex	Male	38	88.4
	Female	5	11.6
Primary tumor site	Buccal mucosa	27	62.8
	Tongue	10	23.3
	Gingivobuccal sulcus	5	11.6
	Palate	1	2.3
Histological grade	Grade 1 (well differentiated)	13	30.2
	Grade 2 (moderately differentiated)	11	25.6
	Grade 3 (poorly differentiated)	19	44.2
Clinical stage	Stage I	7	16.3
	Stage II	3	7.0
	Stage III	8	18.6
	Stage IV	13	30.2
	Stage V	1	2.3
	Not available	11	25.6

TABLE 1: Clinicopathological characteristics of the study population (n=43)

HER2/neu immunohistochemical expression

Immunohistochemical staining for HER2/neu revealed the absence of membranous staining in all 43 cases. None of the tumor samples demonstrated complete or incomplete membranous positivity in malignant epithelial cells. Occasional nonspecific cytoplasmic staining was observed in some sections and was not considered positive.

Controls

The positive control tissue (breast carcinoma) showed appropriate strong membranous staining for HER2/neu (Figure *1*), while the negative control sections showed no staining. This confirmed the technical adequacy and validity of the immunohistochemical procedure (Table *2*).

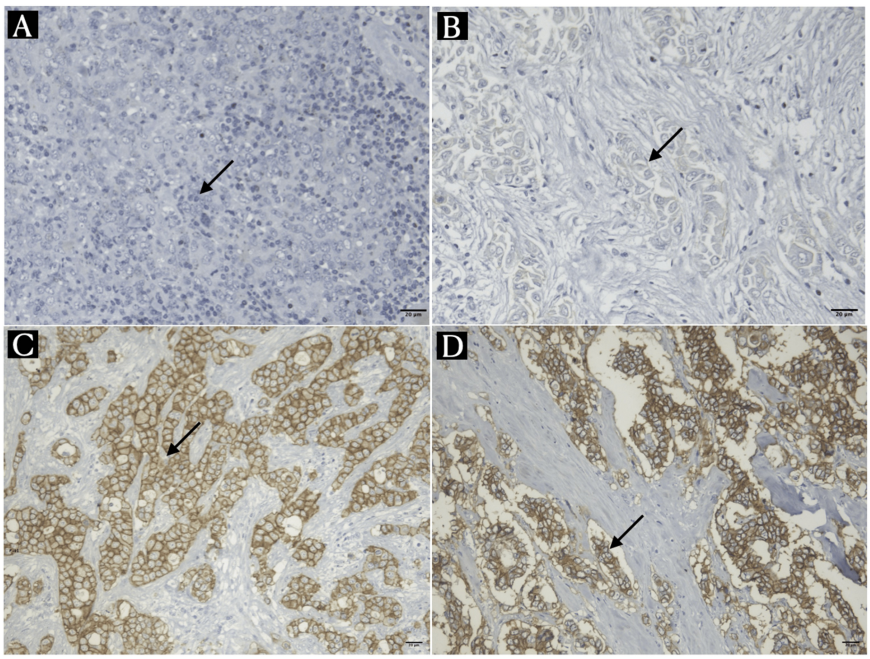


FIGURE 1: Images of breast carcinoma (positive control) showing different levels of positive staining with HER2/neu antibody

Image A shows no positivity, image B shows mild positivity, image C shows moderate positivity, and image D shows high positivity (black arrow showing the stained area).

HER2/neu - human epidermal growth factor receptor-2

HER2/neu expression	n (%)
Positive	0 (0%)
Negative	43 (100%)

TABLE 2: HER2/neu immunohistochemical expression

HER2/neu - human epidermal growth factor receptor-2

Correlation with clinicopathological parameters

As HER2/neu expression was uniformly negative in all cases, no correlation could be established with clinicopathological parameters such as age, sex, tumor site, grade, or stage (Figure *2*).

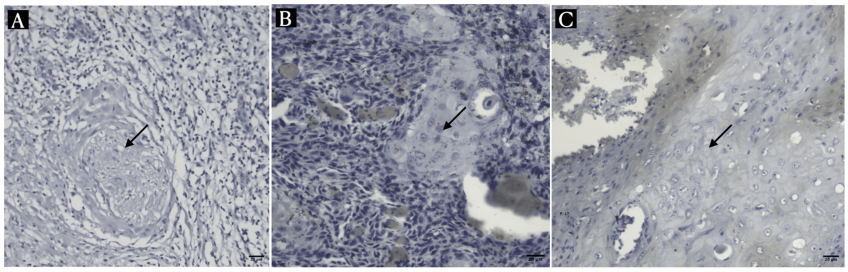


FIGURE 2: Images of oral squamous cell carcinoma showing no positivity with HER2/neu antibody (black arrow showing cancer area)

HER2/neu - human epidermal growth factor receptor-2

Discussion

The present study evaluated the immunohistochemical expression of HER2/neu in 43 cases of oral squamous cell carcinoma (OSCC) and demonstrated a complete absence of membranous HER2/neu expression in all cases. These findings indicate that detectable HER2/neu membranous overexpression was not observed in the study cohort. However, the absence of immunohistochemical expression in this relatively small, single-center sample does not establish the overall biological or prognostic significance of HER2/neu in OSCC. Further studies involving larger and more diverse patient populations, along with molecular assessment of HER2 gene amplification, are necessary to clarify its potential role.

HER2/neu is a well-established prognostic and predictive biomarker in breast and gastric carcinomas, where overexpression identifies patients who may benefit from targeted therapy. However, its role in OSCC is controversial, as published studies have reported highly variable expression rates. All the tumor samples in this study were HER2/neu negative. This is in agreement with previous reports of absent or very low HER2/neu expression in OSCC [6,8].

Mirza et al. found HER2/neu to be positive in only 1 of 140 cases of OSCC and stated that HER2-targeted therapy is unlikely to be of significant benefit to most patients with OSCC. Similarly, Hanken et al. found HER2 overexpression and gene amplification to be rarely detected in OSCC, questioning its value as a clinically useful biomarker [6,8]. These findings are consistent with the results of the present study, in which no HER2/neu membranous expression was detected in the 43 OSCC cases examined. However, the diagnostic and therapeutic implications of HER2/neu expression in OSCC cannot be determined from the present study alone (Table 3).

Study	Country	Sample size	HER2/neu positivity	Main finding
Papavasileiou et al., 2009 [12]	Greece	41	Low	Positive staining was mainly associated with chromosome 17 polysomy rather than HER2 amplification.
Hanken et al., 2014 [8]	Germany	94	Rare	HER2 overexpression and gene amplification were uncommon in OSCC.
Mirza et al., 2020 [6]	Pakistan	140	1/140 (0.7%)	HER2 expression was extremely low; targeted therapy is unlikely to benefit most patients.
Present study	India	43	0/43 (0%)	Complete absence of HER2/neu expression, supporting limited clinical utility in OSCC.

TABLE 3: Comparison of the present study with previous studies evaluating HER2/neu expression in OSCC

HER2/neu - human epidermal growth factor receptor-2; OSCC - oral squamous cell carcinoma

On the other hand, few investigators have reported increased positivity of HER2/neu in OSCC. Previous studies suggested that oral cancers exhibited variable immunoreactivity to HER2/neu, indicating that HER2

alterations may occur in a limited subset of patients [9,10]. These discrepancies could be explained by differences in the antibody clones, tissue fixation, antigen retrieval methods, interpretation criteria, tumor subsites, sample size, and ethnic differences among the study populations. In addition, unlike breast carcinoma, there is no universally accepted HER2 scoring system for OSCC, making comparison between studies difficult [11].

The present study has certain limitations. The relatively small sample size and single-center design may limit the generalizability of the findings. Furthermore, HER2 gene amplification was not evaluated using fluorescence in situ hybridization, and therefore, the possibility of underlying genetic alterations cannot be excluded. The absence of HER2/neu membranous expression in all 43 cases should be interpreted within the context of the study cohort and should not be extrapolated to the entire OSCC population. Larger multicentre studies involving diverse patient populations, standardized immunohistochemical scoring criteria, and molecular validation are warranted to establish the potential diagnostic, prognostic, and therapeutic relevance of HER2/neu in OSCC.

Conclusions

The present study demonstrated the absence of detectable HER2/neu membranous expression in all 43 cases of oral squamous cell carcinoma. These findings indicate that HER2/neu overexpression was not observed in the study cohort. However, the results should be interpreted cautiously considering the relatively small sample size and single-center design. The findings do not establish the overall diagnostic, prognostic, or therapeutic significance of HER2/neu in OSCC. Larger multicentre studies incorporating standardized immunohistochemical assessment and molecular validation, particularly HER2 gene amplification analysis, are necessary to further clarify its potential clinical relevance in OSCC.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Pradheep Kumar KC, Sayan Biswas, Sanjukta Sahoo, Manisha R. Gaikwad

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. All India Institute of Medical Sciences (AIIMS), Bhubaneswar issued approval IEC/AIIMS BBSR/PG THEISIS/2022-23/46. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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