Protocol for evaluation and correlation of CD44 immunoexpression in tumor tissue and surgical margins of oral squamous cell carcinoma with three-year survival: A retrospective study [version 1; peer review: awaiting peer review]

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Abstract

Introduction: The commonest type of cancer in the head and neck region is oral squamous cell carcinoma (OSCC) due to its high rates of occurrence and mortality. Cluster of differentiation 44 (CD44) is a surface glycoprotein present on tumor cells. It regulates cell proliferation, adhesion, migration an invasion of cancer stem cell. Immunoexpression of CD44 in surgical margins indicates poor prognosis of disease and increased risk of local recurrence.[4]

Objectives: To evaluate and correlate CD44 immunoexpression in tumor tissue and surgical margins OSCC with three-year survival.

Methodology: All tissue sections will be processed for CD44 immunohistochemistry. After that, light microscopic evaluation of CD44 expression will be done. A dark brown color of membranous staining of neoplastic cells will be considered for CD44 positivity; this must be observed in the tumor core, in the basal layer because most stem cells are present in the basal layer of oral mucosa and in the invasive front of tumor.

Expected results: The present study will find the immunohistochemical expression of CD44 tumor tissue and surgical margins of OSCC, in order to evaluate the prognosis of OSCC. Immunoexpression may vary in according to their different grades.
Conclusions: We hypothesize that, as the disease stage advances, intensity of CD44 expression immunohistochemically increases and it can adversely affect overall survival; hence it is considered as an independent predictor of prognosis of OSCC.

Keywords
CD44, CSCs, Oral squamous cell carcinoma, surgical margins of OSCC, Immunohistochemistry

This article is included in the Cell & Molecular Biology gateway.

This article is included in the Oncology gateway.

This article is included in the Developmental Psychology and Cognition gateway.
**Introduction**

**Background and rationale**

The commonest type of cancer in head and neck region is oral squamous cell carcinoma (OSCC), due to its high rates of occurrence and mortality. More than 90% of cases of oral and oropharyngeal cancer are OSCC cases. Although OSCC treatment techniques (surgery, radiation, chemotherapy) have improved, survival has not. The overall five-year survival rate is less than 50% globally.

The squamous epithelia inside the mouth give rise to OSCC. According to the cancer stem cells hypothesis, a tumor’s core contains a subpopulation of multipotent cells that cause tumor differentiation, continuation, and its metastasis to other places. Cancer stem cells (CSCs) are thought to be resistant to conventional therapy and are capable of producing new tumor cells that genetically resembles the original tumor. The ability of CSCs to regenerate themselves causes recurrence of disease. We must find and eliminate these self-renewing cells in order to improve survival.

Identification of these cells is done by detecting expression of CSCs markers which are present either on their surfaces or within the cells. There are various cancer stem cell markers such as CD44, CD133, L1CAM and SOX2.

Cluster of differentiation 44 (CD44) is a surface glycoprotein present on tumor cells. It regulates cell proliferation, adhesion, migration and invasion of cancer stem cell. Various studies have reported that CD44 immunoexpression is an independent predictor for prognosis.

In order to improve prognosis, surgical removal of tumor should be done with adequate negative surgical margins. Immunoexpression of molecular markers in surgical margins indicates poor prognosis of disease and increased risk of local recurrence.

According to a literature search, no study has been done on CD44 expression in surgical margins of OSCC; therefore, we aim to assess immunoexpression of CD44 in surgical margins of OSCC, in order to evaluate its relationship with histologic types and overall survival.

**Objectives**

1. To evaluate immunoexpression of CD44 in various grades of OSCC.
2. To evaluate immunoexpression of CD44 in surgical margins of various grades of OSCC.
3. To compare immunoexpression of CD44 in tumor tissue and surgical margins of different grades of oral squamous cell carcinoma.
4. To correlate CD44 immunoexpression with histopathological grading of OSCC.
5. To relate CD44 immunoexpression to three-year survival of OSCC patients.

**Trial design**

Retrospective cohort study.

**Methods**

**Study setting**

This study will be conducted in the Department of Oral Pathology and Microbiology, Sharad Pawar Dental College in collaboration with Central Research Laboratory, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe) Wardha.

**Eligibility criteria**

The inclusion criteria of this study will comprise of 80 surgically treated patients who have been clinically and histopathologically diagnosed with OSCC. Follow-up data will be kept in record for disease-free survival for three years before the study starts. The cases that will be excluded from the studies who previously underwent surgery for recurrence, had a history of oral cancer, recurring or distant disease and underwent preoperative chemotherapy, or radiation therapy.
**Intervention**

Paraffin-embedded tissue blocks having suitable mass of tumor and acceptable amount of normal tissue will be cut into 4-μm thick tissue pieces. All tissue sections will be processed for CD44 immunohistochemistry. After that, light microscopic evaluation of CD44 expression will be done. A dark brown color of membranous staining of neoplastic cells will be evaluated for CD44 positivity which have to be observed in the tumor core, in the basal layer, because most of stem cells are present in the basal layer of oral mucosa and in the invasive front of tumor.

**Outcomes**

*Primary outcome*

The present study will measure the immunohistochemical expression of CD44 tumor tissue and surgical margins of OSCC, in order to evaluate the prognosis of OSCC.

*Secondary outcome*

Immunooxpression of CD44 may vary in different grades of OSCC and accordingly affect the overall and disease-free survival.

**Sample size**

Considering the prevalence of OSCC as 70.7% in the outpatient department of Oral Pathology and Microbiology, Sharad Panwar Dental College and Hospital, using the single proportion formula (Angadi et al., 2007), the sample size is calculated by applying the formula:

\[
n \geq \frac{Z_{1-\alpha/2}^2 \cdot p \cdot (1-p)}{d^2}
\]

Where

\[Z_{1-\alpha/2}^2\] The significance level at five (5)%

\[i.e. 95\% \text{ confidence interval} = 1.96\]

\[p\] - Sample showing positive CD 44 expression focally in small group cells in the basal layer of epithelium = 70.7% = 0.707

\[E\] - Error of margin = 10% = 0.10

\[
n = 1.96^2 \times 0.707 \times (1 - 0.707)/0.10^2
\]

\[n = 80\]


**Immunostaining**

Paraffin-embedded tissue blocks will be cut into 4 μm thick tissue pieces (fixed with formalin). They will be transferred to glass slides made of 3-amino propyl triethoxy silane (APES). The slides will be deparaffinized with xylene and rehydrated with reduced ethanol concentrations. The slides will be heated in TRIS-EDTA for five minutes in a pressure boiler to perform antigen retrieval. With 3% H_2O_2, endogenous peroxidase activity will be blocked for 10 minutes. The plates will be exposed to ultraviolet block reagent for five minutes. Slides will be incubated with the main CD44 antibody for one hour at room temperature. After that, the secondary antibody will be added for 30 minutes with horse radish peroxidase. 3'-diaminobenzidine tetrahydrochloride will be used as the substrate chromogen for staining, and Harris Hematoxylin will be used for counterstaining. Sections will first be dehydrated, then cleared, mounted, and finally observed under a light microscope.

**Dissemination**

The resulting research article will be published in an indexed journal.
Study status
The study has not started yet.

Discussion
Kaza et al. (2018) investigated the immunoexpression of CD44 in OSCC and its relationship to tumor histological grading on 10 tissue specimens. When compared to the normal oral mucosa in this investigation, a drop in immunoexpression of CD44 with increasing grades of OSCC was seen. The findings of this study point to altered CD44 immunoexpression in OSCC and weak immunostaining in squamous cell carcinomas with low differentiation. Therefore, it may be concluded that the absence of cell adhesion, associated with the reduction in CD44 expression, may be useful in predicting the development of OSCC.8

Boxberg et al. (2018) assessed CD44’s impact on OSCC in correlation with histomorphologic parameters, clinicopathological factors and their effect on patient prognosis. They conducted a cohort study of 108 OSCC patients that had never received therapy with thorough long-term follow-up, and they concluded that CD44 was associated with combativeness of tumor and epithelial-mesenchymal transition as well as having an independent prognostic impact in a subgroup of OSCCs. This highlights the importance of tumor cell stemness as a principle factor in the malignant action of this disease.4

Ranka et al. (2020) investigated molecular markers in surgical margins of OSCC with negative surgical margins in order to increase patient survival. They also examined the predictive value of immunohistochemistry markers in surgical margins of OSCC on Indian patients. They came to the conclusion that using immunohistochemistry markers to identify surgical margins free of tumor cells might reduce recurrence of OSCC and increases survival.6

According to Suresh et al’s (2019) study on prognostic indicators of OSCC, females aged less than 65 years having tongue and alveolus lesions, the early T Stage and N0 with negative margin, and it had a significant positive impact on disease-free and overall survival of the oral cancer patients.9

Ethical considerations
Ethical approval received from Datta Meghe Institute of Higher Education and Research, Sawangi, Wardha (IEC reference number DMIHER (DU)/IEC/2023/841).

Data availability
Underlying data
No data is associated with this article.

Reporting guidelines

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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References


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