Research Article

Immunohistochemical Expression of SOX2 and OCT4 in Oral Squamous Cell Carcinoma: A Pilot Study

Sajda Khan
Department of Oral Pathology and Microbiology, People's Dental Academy, People's University, Bhopal (Madhya Pradesh)

ABSTRACT:

Background: Oral squamous cell carcinoma (OSCC) is one of the most frequently-occurring malignancies of the oral cavity and continues to be associated with poor prognosis, recurrence and metastasis despite improvements in diagnosis and treatment. Cancer stem cells (CSCs) are thought to play an important role in the process of tumour initiation, progression, invasion and resistance to therapy. SOX2 and OCT4 are two significant transcription factors of the embryo stem cells that play a role in the maintenance of the pluripotency and self-renewal of the ESCs. These markers have been found to be over-expressed in various cancers and may be associated with the aggressive behaviour of the tumour.

Objective: To evaluate the immunohistochemical expression of SOX2 and OCT4 in OSCC and to correlate the expression with clinicopathological parameters.

Materials and Methods: The present observational pilot study was conducted on 10 formalin fixed paraffin embedded (FFPE) tissue blocks that were used to retrieve 10 cases of oral squamous cell carcinoma (OSCC) with histopathological confirmation. SOX2 and OCT4 antibody was used for immunohistochemical staining. Nuclear brown staining was deemed as positive. Semi-quantitative scoring was performed based on the percentage of positive cells: 0–5% (1+), 6–25% (2+), 26–50% (3+), and >50% (4+). Descriptive statistics and Chi-square test were used for statistical analysis.

Results: All the cases of OSCC in this study expressed the proteins SOX2 and OCT4 positively by immunohistochemistry. As the grade increased, the expression of SOX2 and OCT4 increased. The immunoreactivity in poorly differentiated OSCC was higher than that in well-differentiated lesions.

Conclusion: The present pilot study showed that SOX2 and OCT4 expression was found to be upregulated in OSCC. The increased expression of these CSC markers could be associated with tumor progression, invasion, and prognosis. SOX2 and OCT4 could be helpful prognostic markers and therapeutic targets in OSCC.

KEY WORDS: SOX2, OCT4, Oral Squamous Cell Carcinoma, Cancer Stem Cells, Immunohistochemistry, Oral Cancer

Address for correspondence : Dr. Sajda Khan, PhD Scholar, Department of Oral Pathology and Microbiology, People's Dental Academy, People's University, Bhopal (Madhya Pradesh) , India, E-mail: drsajdagajdhar@gmail.com
Submitted: 04.01.2026, **Accepted:** 02.06.2026, **Published:** 24.06.2026

INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant oral disease and has contributed over 90% of all oral cancers. In the countries of South Asia, the burden of OSCC is high with the prevalence of chewing of tobacco, smoking of tobacco, alcohol and areca nut. Although surgical methods, radiotherapy, and chemotherapy have been improved over the past several decades, the overall survival rate of the OSCC patients has not significantly improved.[1-3]

Access this article online

Quick Response Code:

Website: www.pjsr.org

DOI: https://doi.org/10.5281/zenodo.20828124

How to cite this article: Khan S. Immunohistochemical Expression of SOX2 and OCT4 in Oral Squamous Cell Carcinoma: A Pilot Study. PJSR. 2026;19(1):07-12.

OSCC is a multistep process that occurs through genetic and epigenetic changes, and culminates in the development of an invasive carcinoma from normal oral epithelium. The occurrence of tumour heterogeneity and recurrence has prompted the study of cancer stem cells (CSCs) in oral carcinogenesis. CSCs are a small subset of tumor cells that exhibit self-renewal capacity, differentiation potential and resistance to conventional therapies. [4]

Many CSC markers have been noted, including Sex Determining Region-Y Box 2 (SOX2), and Octamer-Binding Protein 4 (Oct4). The transcription factor SOX2 is a regulator of pluripotency and embryonic stem cell self-renewal. It is a member of SOX family of transcription factors and has been found to be involved in the initiation of tumors, epithelial-mesenchymal transition, invasion and metastasis. [5-10]

Another key transcription factor that plays a role in maintaining stemness and self-renewal in embryonic stem cells is OCT4 (Octamer-binding transcription factor-4), also called as POU5F1. Hyper-expression of OCT4 has been observed in various cancers including oral, and associated with tumour aggressiveness and poor prognosis.[6,7]

It has been found that the expression of SOX2 and OCT4 are upregulated in OSCC. But there are very few studies that have comparatively assessed these markers in OSCC and related them with clinicopathological parameters. Hence, the present **pilot** study was designed to assess the expression of SOX2 and OCT4 in OSCC cases.[6-8]

AIM AND OBJECTIVES:-

Aim: To evaluate the immunohistochemical expression of SOX2 and OCT4 in Oral Squamous Cell Carcinoma.

Objectives:

1. To assess the immunohistochemical expression of SOX2 in OSCC.
2. To assess the immunohistochemical expression of OCT4 in OSCC.
3. To correlate SOX2 and OCT4 expression with histopathological grading of OSCC.

MATERIALS & METHODS:

Study Design **:** The present study was a retrospective observational pilot study.

Study Sample**:** A total of 10 histopathologically confirmed cases of OSCC were retrieved from the archival records of the Department of Oral Pathology.

Inclusion Criteria**:** Histopathologically confirmed cases of OSCC, adequate tissue available in paraffin blocks and complete clinicopathological details available.

Exclusion Criteria**:** Tissue blocks with inadequate tissue, poorly preserved tissue specimens and recurrent OSCC cases.

Study Setting:

This research was conducted as a pilot study forming part of the PhD thesis entitled "Immunohistochemical Expression of SOX2 and OCT4 in Oral Squamous Cell Carcinoma." The purpose of the pilot study was to assess the feasibility of the study design, optimize immunohistochemical protocols, and generate preliminary data prior to undertaking the full-scale thesis research. Ethical approval was obtained for the study. Formalin-fixed paraffin-embedded tissue samples were collected from the archives of the Department of Oral Pathology and microbiology, People's Dental Academy. Immunohistochemical staining and analysis for SOX2 and OCT4 were performed in a specialized diagnostic laboratory in Mumbai.

Duration of the Study:

The study was conducted over a period of one year, which included case selection, retrieval of tissue blocks, immunohistochemical processing, microscopic evaluation, statistical analysis, and interpretation of the results.

Immunohistochemical Procedure:

Formalin fixed paraffin embedded tissue blocks were cut into sections of 3-4 µm thickness on a semi-automated microtome. One section underwent Hematoxylin and Eosin staining and the other one was stained by the immunohistochemistry with SOX2 and OCT4 antibody.

The sections were deparaffinized with xylene and rehydrated with descending grades of alcohol. Antigen retrieval was done with trisodium citrate buffer (pH 6). To inhibit endogenous peroxidase activity, 3% hydrogen peroxide was used. The primary anti-SOX2 and anti-OCT4 antibodies were used, and incubated in a humid chamber. Then Post Primary Reagent and the reagent-Polymer (Polymer HRP) were added. The chromogen used was Diaminobenzidine (DAB) and the counterstain was hematoxylin.

Brown nuclear staining was taken as being positive for both markers.

Evaluation of Immunostaining:

Ten to twelve representative high power (400×) fields were scanned for positive cells. The percentage of positive cells was graded semi-quantitatively as follows:

TABLE 1: Clinicopathological Details of OSCC Cases and expression of SOX2 and OCT4

Case No.	Age	Gender	Site	Histological Grade	TNM Stage	SOX2 Expression	OCT4 Expression
1	45	Male	Buccal mucosa	Well differentiated	Stage I	2+	2+
2	52	Male	Tongue	Well differentiated	Stage I	2+	2+
3	60	Male	Buccal mucosa	Moderately differentiated	Stage II	3+	3+
4	49	Female	Tongue	Moderately differentiated	Stage II	3+	3+
5	55	Male	Buccal mucosa	Moderately differentiated	Stage III	3+	4+
6	62	Male	Alveolus	Poorly differentiated	Stage IV	4+	4+
7	58	Male	Buccal mucosa	Poorly differentiated	Stage IV	4+	4+
8	47	Female	Tongue	Well differentiated	Stage I	2+	2+
9	64	Male	Buccal mucosa	Moderately differentiated	Stage III	3+	3+
10	51	Male	Buccal mucosa	Well differentiated	Stage II	2+	3+

TABLE 2: Distribution of SOX2 and OCT4 Expression According to Histopathological Grades.

Histopathological Grade	Number of Cases	Mean SOX2 Expression	Mean OCT4 Expression	*p* value
Well Differentiated OSCC	4	2+	2+	
Moderately Differentiated OSCC	4	3+	4+	<0.05*
Poorly Differentiated OSCC	2	4+	4+	

*Statistically significant

Percentage of Positive Cells	Score
0–5%	1+
6–25%	2+
26–50%	3+
>50%	4+

Statistical Analysis:

Data were analyzed using descriptive statistics. Mean and percentage values were calculated. Chi-square test was used to assess correlation between marker expression and clinicopathological parameters. *p*-value <0.05 was considered statistically significant.

RESULTS:

A total of 10 cases of oral squamous cell carcinoma (OSCC) were included in this pilot study. The clinicopathological characteristics of all cases, including age, gender, anatomical site, histopathological grade, TNM stage, and immunohistochemical expression of SOX2 and OCT4, are summarized in Table 1.

Demographic and Clinicopathological Characteristics:

Among the 10 patients, 8 (80%) were males and 2 (20%) were females, yielding a male-to-female ratio of 4:1. The majority of patients were older than 40 years of age, indicating a higher prevalence of OSCC in the middle-aged and elderly population.

The buccal mucosa was the most frequently involved site, followed by the tongue and alveolus. Histopathological examination revealed that 4 cases (40%) were well-differentiated OSCC, 4 cases (40%) were moderately differentiated OSCC, and 2 cases

(20%) were poorly differentiated OSCC.

***Immunohistochemical Expression of SOX2 and OCT4*:**

Positive immunohistochemical expression of both SOX2 and OCT4 was observed in all OSCC cases. However, the intensity and extent of staining varied according to the histopathological grade.

As shown in Table 2, the expression of both SOX2 and OCT4 demonstrated a progressive increase from well-differentiated to moderately differentiated and poorly differentiated OSCC. Poorly differentiated tumors exhibited the highest levels of marker expression, whereas well-differentiated tumors showed comparatively lower expression.

Statistical analysis using the Chi-square test revealed a significant association between the expression levels of SOX2 and OCT4 and the histopathological grade of OSCC ($p < 0.05$). This finding suggests that increased expression of these cancer stem cell markers is associated with decreasing tumor differentiation and greater biological aggressiveness.

The most notable finding of this pilot study was the universal positive expression of both SOX2 and OCT4 in all OSCC cases. A progressive increase in the expression of these markers was observed from well-differentiated to poorly differentiated tumors, with the highest levels seen in poorly differentiated OSCC. Statistical analysis demonstrated a significant association between SOX2 and OCT4 expression and histopathological grade ($p < 0.05$). These preliminary results suggest that SOX2 and OCT4 may serve as potential biomarkers of tumor aggressiveness and could have prognostic value in oral squamous cell carcinoma.

DISCUSSION:

Oral squamous cell carcinoma (OSCC) is one of the most aggressive malignancies of the oral cavity, and a significant cause of cancer related morbidity and mortality worldwide. Although the OSCC diagnosis and therapeutic approaches have undergone significant enhancements in recent decades, the rate of survival for patients with OSCC has not improved significantly. Tumor recurrence, metastasis, therapeutic resistance and tumor heterogeneity are among the key factors that contributes to poor prognosis. The ability of CSCs to initiate, progress, invade, metastasize and be resistant to therapies, has made them an important target of cancer research over the past few years.[1-4,11-20]

Cancer stem cell (CSC) is a tiny fraction of tumor cells, with characteristics of both self-renewal and differentiation that resemble those of embryonic stem cells. It is thought these cells contribute to the maintenance of a tumour and recurrence post treatment. A number of molecular markers have been examined to detect CSCs in oral cancer and SOX2, OCT4 are regarded as important pluripotency-associated transcription factors.[4,5]

SOX2 belongs to the sex-determining region Y-box transcription factor family and is a key factor in the maintenance of pluripotency and stemness of embryonic stem cells. Also, POU5F1 encodes a transcription factor known as OCT4, which is regarded as a master regulator of stem cell self-renewal and differentiation. The markers are often found to be dysregulated in breast carcinoma, lung carcinoma, colorectal carcinoma, gastric carcinoma, pancreatic carcinoma and oral squamous cell carcinoma.[10,11,17-21]

In the present pilot study, the immunohistochemical expression of SOX2 and OCT4 was evaluated in 10 cases of OSCC, confirmed by the histopathologic analysis. In all the cases studied, both markers were expressed positively. Both SOX2 and OCT4 were judged to be 'positive' with nuclear brown staining.

In current study, most of the OSCC cases were seen in males, and those older than 40 years. The most frequent site of involvement was buccal mucosa. This is similar to previous studies that reported that male had a higher prevalence of OSCC as they are reportedly exposed to more tobacco, smoking and areca nut chewing, and alcohol consumption.[1-3]

In the present study, we found that SOX2 is being increasingly expressed in well differentiated OSCC compared to poorly differentiated OSCC. In poorly differentiated cases, the tumor islands were characterized by strong nuclear positivity, and cells were diffusely distributed throughout the islands with SOX2 nuclear expression. The staining was mainly found in the periphery of the tumor and in the tumor fronts in well differentiated lesions.

These observations are consistent with those obtained by Du et al. and Qiao et al., who observed higher levels of SOX2 expression in the late stages of OSCC. Du et al. found that the expression of nuclear SOX2 was significantly correlated with poor prognosis in oral tongue squamous cell carcinoma and proposed that SOX2 may be used to identify a novel independent prognostic marker. The SOX2 expression was observed to be gradually increased from well differentiated to poorly differentiated oral squamous cell carcinoma, suggesting its role in progression of oral carcinogenesiS.[5,6]

Murakami et al. found that there was a

significant positive correlation between the expression of SOX2 and the progression of OSCC and poor disease-specific survival, when they increased its expression in the basal layers of OSCC. Likewise, Mazibrada et al. showed an increasing expression of SOX2 during the progression of the oral carcinogenesis and its highest expression in poorly differentiated squamous cell carcinoma.[9]

Overexpression of SOX2 in poorly differentiated OSCC as observed in the present study may suggest an increased level of cancer stem cell activity in aggressive tumors. SOX2 has been described to control the proliferation of tumors, EMT, invasion and metastatic properties via various signaling pathways such as Wnt/β-catenin signaling.[10,17]

The present study also showed positive immunohistochemical expression of OCT4 in all cases of OSCC. Like SOX2, OCT4 was also found to increase with the progression of the histopathological grades. Poorly differentiated OSCC cases showed strong and diffuse nuclear staining while the well differentiated cases showed mild to moderate staining. The results are consistent with a previous study by Fu et al. who found that there was significant correlation between the expression of OCT4 and the progression of OSCC. Overall, the authors proposed that the maintenance of OCT4 expression may play a role in the stemness and tumor aggressiveness of OSCC.[7]

Zisis et al. also studied SOX2 and OCT3/4 expression in OSCC and oral leukoplakia, and reported that SOX2 and OCT3/4 were expressed more in OSCC than in potentially malignant disorders. They highlighted the importance of oral cancer markers generated from embryonic stem cells in oral carcinogenesis and progression.[8]

This up-regulation of OCT4 in poorly differentiated tumors in the present study suggests that aggressive tumors have more proliferative and stem cell-like characteristics. OCT4 has shown to have pro-survival effects, resistance to chemotherapy and radiation therapy, and self-renewal properties.[6,7]

In the present study, the simultaneous overexpression of SOX2 and OCT4 in OSCC corroborates the hypothesis that both these markers exert a synergistic effect in retaining cancer stem cell phenotype. Cancer stem cells are able to resist the treatment and regenerate tumor tissue, which may lead to recurrence and metastasis.[4,7]

The involvement of SOX2 and OCT4 in various kinds of cancer has been reported. Herreros-]Villanueva et al. showed that SOX2 is a promoter of dedifferentiation and stem cell-like properties in pancreatic carcinoma. Tang et al. found that SOX2 regulates EMT and cancer stemness in colorectal carcinoma by Wnt / β-catenin pathway. Similarly, Hushmandi et al. and Dey et al. have reported the diverse functions of SOX2 in breast and lung cancer.[11,17-19]

CONCLUSION:

In the present pilot study, it has been shown that the expressions of SOX2 and OCT4 are positive in Oral Squamous Cell Carcinoma. The expression of these markers was correlated with the advanced histopathological grade.

Results revealed that SOX2 and OCT4 might be vital to the maintenance of cancer stem cell properties and therefore to tumor progression and aggressiveness. These markers could be used as possible prognosis biomarkers and future therapeutic targets for OSCC.

Identification and examination of these cancer stem cell markers offers a wide range if possibilities for targeted killing of these populations, potentially contributing to the cessation of tumor growth and prevention of disease recurrence.

It requires analysis of these CSC markers in a larger cohort of patients with OSCC to reveal its potential diagnostic, prognostic, and predictive value.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

REFERENCES:

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
2. Bhat R, Karicheri AB, Palakki G, Uday M. Antibody-Drug Conjugates: Advances and Applications in Targeted Cancer Therapies. Oral Sphere J. Dent. Health Sci. 2025;1(2):63-72. DOI: 10.63150/osjdhs.2025.3
3. Vigneswaran N, Williams MD. Epidemiologic trends in

head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am. 2014 May;26(2):123-41. doi: 10.1016/j.coms.2014.01.001. PMID: 24794262; PMCID: PMC4040236.

4. Kaur T, Khan N, Pasha Z et al. Stem Cells: Innovations, Applications, and Future Directions. J Pharm Bioallied Sci. 2024 Dec;16(Suppl 4):S3041-S3043. doi: 10.4103/jpbs.jpbs_1089_24. Epub 2024 Nov 13.
5. Du L, Yang Y, Xiao X, Wang C, Zhang X, Wang L, Zhang X, Li W, Zheng G, Wang S, Dong Z. Sox2 nuclear expression is closely associated with poor prognosis in patients with histologically node-negative oral tongue squamous cell carcinoma. Oral Oncol. 2011 Aug;47(8):709-13. doi: 10.1016/j.oraloncology. 2011.05.017. PMID: 21689966.
6. Qiao B, He B, Cai J, Yang W. The expression profile of Oct4 and Sox2 in the carcinogenesis of oral mucosa. Int J Clin Exp Pathol. 2013 Dec 15;7(1):28-37. PMID: 24427323; PMCID: PMC3885457.
7. Fu TY, Hsieh IC, Cheng JT, Tsai MH, Hou YY, Lee JH, Liou HH, Huang SF, Chen HC, Yen LM, Tseng HH, Ger LP. Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression. J Oral Pathol Med. 2016 Feb;45(2):89-95. doi: 10.1111/jop.12335. Epub 2015 Jul 25. PMID: 26211876.
8. Zisis V, Andreadis D, Anastasiadou PA, Akrivou M, Vizirianakis IS, Anagnostou L, Malamos D, Paraskevopoulos K, Poulopoulos A. Expression of the Embryonic Cancer Stem Cells' Biomarkers SOX2 and OCT3/4 in Oral Leukoplakias and Squamous Cell Carcinomas: A Preliminary Study. Cureus. 2023 Sep 18;15(9):e45482. doi: 10.7759/cureus.45482. PMID: 37859926; PMCID: PMC10584277.
9. Murakami K, Kamiakito T, Kawata H, Kosami K, Nakaya T, Noguchi T, Mori Y, Tanaka A. Basal differentiation and expression status of SOX2 and KLF4 in basal layers are prognostic factors for disease-specific survival in oral squamous cell carcinoma. J Oral Pathol Med. 2024 May;53(5):303-309. doi: 10.1111/jop.13535. Epub 2024 Apr 24. PMID: 38659289.
10. Ding LN, Yu YY, Ma CJ, Lei CJ, Zhang HB. SOX2-associated signaling pathways regulate biological phenotypes of cancers. Biomed Pharmacother. 2023 Apr;160:114336. doi: 10.1016/j.biopha.2023.114336. Epub 2023 Feb 2. PMID: 36738502.
11. Herreros-Villanueva M, Zhang JS, Koenig A, Abel EV, Smyrk TC, Bamlet WR, de Narvajas AA, Gomez TS, Simeone DM, Bujanda L, Billadeau DD. SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells. Oncogenesis. 2013 Aug 5;2(8):e61. doi: 10.1038/oncsis.2013.23. PMID: 23917223; PMCID: PMC3759123.
12. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007 Nov;36(10):575-80. doi: 10.1111/j.1600-0714.2007.00582.x. PMID: 17944749.
13. Bhargava A, Saigal S, Chalishazar M. Histopathological grading systems in oral squamous cell carcinoma: A review. J Int Oral Health. 2010;2(4):1–10. https://api.semanticscholar.org/CorpusID:29833218.
14. Greene FL, Page DL, Fleming ID, et al. AJCC Cancer Staging Manual. 6th ed. Springer; 2002.pp.1-195. https://www.facs.org/media/tauiudl3/ajcc_6thed_cancer_staging_manual_part1.pdf
15. Velcheti V, Schalper K, Yao X, Cheng H, Kocoglu M, Dhodapkar K, Deng Y, Gettinger S, Rimm DL. High SOX2 levels predict better outcome in non-small cell lung carcinomas. PLoS One. 2013 Apr 19;8(4):e61427. doi: 10.1371/journal.pone.0061427. PMID: 23620753; PMCID: PMC3631238.
16. Sawhney H, Bhargava D, Kashwani R et al Artificial intelligence as a tool for improving oral cancer outcomes.Arch Dent Res 2023;13(1):15-19. | https://doi.org/10.18231/j.adr.2023.003
17. Tang Q, Chen J, Di Z, Yuan W, Zhou Z, Liu Z, Han S, Liu Y, Ying G, Shu X, Di M. TM4SF1 promotes EMT and cancer stemness via the Wnt/β-catenin/SOX2 pathway in colorectal cancer. J Exp Clin Cancer Res. 2020 Nov 5;39(1):232. doi: 10.1186/s13046-020-01690-z. PMID: 33153498; PMCID: PMC7643364.
18. Hushmandi K, Saadat SH, Mirilavasani S, Daneshi S, Aref AR, Nabavi N, Raesi R, Taheriazam A, Hashemi M. The multifaceted role of SOX2 in breast and lung cancer dynamics. Pathol Res Pract. 2024 Aug;260:155386. doi: 10.1016/j.prp.2024.155386. Epub 2024 Jun 8. PMID: 38861919.
19. Dey A, Kundu M, Das S, Jena BC, Mandal M. Understanding the function and regulation of Sox2 for its therapeutic potential in breast cancer. Biochim Biophys Acta Rev Cancer. 2022 Mar;1877(2):188692. doi: 10.1016/j.bbcan.2022.188692. Epub 2022 Feb 2. PMID: 35122882.
20. Khan S, Gupta A. Oral cancer stem cells: A comprehensive review. IOSR Journal of Dental and Medical Sciences. 2026;25(5):49–52. doi:10.9790/0853-250501495
21. Abdou AG, Mostafa AF, Gafar S, Farag AGA. Immunohistochemical expression of SOX2 in non-melanoma skin cancer. J Cosmet Dermatol. 2022 Jun;21(6):2623-2628. doi: 10.1111/jocd.14433. Epub 2021 Sep 8. PMID: 34496119.