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Immunohistochemical expression of SOX2 in Oral Squamous cell carcinoma in Western Gujarat Population

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Abstract:
In this study we aim to investigate the role of SOX2 for monitoring disease progression in Oral Squamous Cell Carcinomas which may serve as prognostic marker to predict its recurrence. To investigate the role of SOX2 in Tumour cells of different grades of Squamous cell carcinoma patients.
Method: *21 Tissue samples were collected from the from the Tongue, Buccal Mucosa, Alveolar Mucosa and Lip site from the patients aged between 30 to 70 years. All the samples were histologically confirmed for OSCC.*
Results: *Histologically Different grades of OSCC exhibited positivity for SOX2 significantly. Poorly differentiated squamous cell carcinoma showed higher concentration of SOX2 (85.7%) than the moderately differentiated (42.9%) and well differentiated type (14.3%) respectively.*
Key words: *Oral Squamous Cell Carcinoma, Cancer stem cell markers, Sox2*

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I. INTRODUCTION

Oral cancer is the sixth most common cancer type worldwide with an average yearly incidence of 3 lacs cases and 1.30 lacs death. [1] Oral Cancer is a complex heterogenous disease that develops as a consequence of genetic and epigenetic alterations. The sequential event begins at precancerous stage and control tumour growth. [2] oncogene and tumour proliferative and suppressive genes were highly crucial for development of squamous cell carcinoma. [2]

Individual tumours consist of a mixed cell population that differs in function, morphology and molecular signatures. These tumours reside in and interact with their microenvironment, which consist of wide variety of cell types and cellular structures, such as immune cells, fibroblasts, blood vessels and the extracellular matrix. Tumour cells themselves can be of multiple clonal populations, each having accumulated unique molecular alterations over the course of tumour development and growth. In addition, tumour cells that are similar at the genetic level may have distinct modes of epigenetic regulation, further increasing the functional heterogeneity.

It has been hypothesized that only small subset of tumour cells is capable of initiating and sustaining tumour growth; they have been termed cancer stem cells (CSCs). [3]

CSCs have been isolated from many organs and confirmed to have stem cell like abilities such as self-renewal, multilineage differentiation and expression of stemness- related markers. [4] these cells may also play a role in disease recurrence after treatment and remission. As such targeting of CSCs is currently an active area of therapeutic development. [5]

Embryonic stem cells markers

CSCs in OSCC express the same proteins that regulate the embryonic stem cells (ESCs). OCT4, NANOG, and SOX2 are considered to be the master regulators for self-renewal and maintenance of the stem cell population in the undifferentiated state. Fu *et al*. demonstrated that immunohistochemical expression of OCT4 and SOX2 is significantly higher in tumour-adjacent tissue compared to both normal tissue and the tumour, whereas, NANOG is highly expressed in both tumour and peritumoral tissue, compared to the normal tissue. [6]

SOX2

The SOX2 protein is an SRY-related HMG box transcription factor involved in multiple signal transduction pathways that control cell proliferation, migration, invasion, stemness, tumorigenesis and

anti-apoptosis. SOX2 is known to complex with OCT4, and in murine cell lines has been shown to control the expression of the NANOG gene.[7]

In SCC of the buccal mucosa, SOX2 is expressed within the tumour nests, the peritumoral stroma and the micro-vessels within the peritumoral stroma, similar to OCT4. Fu *et al*. showed that SOX2 expression in OSCC correlated with small tumour size and early tumour stage, and better disease-free survival. Chou *et al*. demonstrated that overexpression of SOX2 enhances the invasiveness and xenotransplantation tumorigenicity in OSCC cells. The study also showed that silencing SOX2 suppresses the expression of drug resistance and anti-apoptotic genes.[8]

II. Methods:

Study Frame: This study was retrospective cohort with a distinguish design of for survival rate. Data were collected from the Oral Pathology department at Government Dental College and Hospital, Jamnagar. Few tissue-blocks and Histopathology slides were retrieved from the patients with verbal and written consent coming to Private practitioner in Jamnagar city and then the record of recurrence rate was recorded

Sample: The data sample of total 21 patients (n= 21) were obtained from the patient record. Only the tissue sample of primary tumour, collected from the patient who reported back to the department for its recurrence during the time frame between 2019 to 2023.

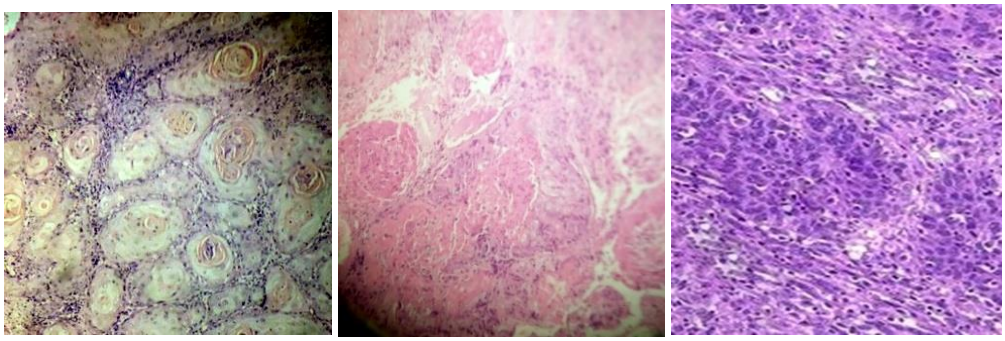
Inclusion criteria were specimens of histologically confirmed Oral Squamous cell carcinomas of different grades between the age of 30 to 70 years. Samples were excluded of the patient if patient did not report for its recurrence.

Immunohistochemical Analysis: The samples were deparaffinized in xylene and processed through an alcohol grading to evaluate the SOX2 concentration. FFPE blocks cut into 3-4 µm sections and kept it to dry at 60° for 3 hours, deparaffinised and dehydrated. Antigen retravel was done by the use of microwave in 10mM sodium citrate buffer pH 6.0 for 20 minutes, followed by peroxide blocking for 30 minutes in dark. The sections then blocked with 5% Bovine serum Albumin (BSA) in Phosphate Buffered Saline (PBS), followed by addition of primary antibody (1:1000) at 4 ° C for overnight. Next day, sections were washed with PBS and then incubated with secondary antibody (1:1000) conjugated with Horseradish Peroxidase (HRP) followed by washing and treatment with DAB chromogen (Diaminobenedine). Afterwards, sections were washed and counterstained with Haematoxylin. Prepared slides were observed under Bright-field microscopy imaging. Histoscore was recorded in the samples of different histological grades of Squamous cell carcinomas. Histoscore was calculated by the formula [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)] using image J software; where percentage of positive cells (0–100%) were multiplied with the intensity (weak: 1, moderate: 2 and strong: 3) to obtain a maximum score of 300. In Sox2, positive markings were calculated based on nuclear staining.

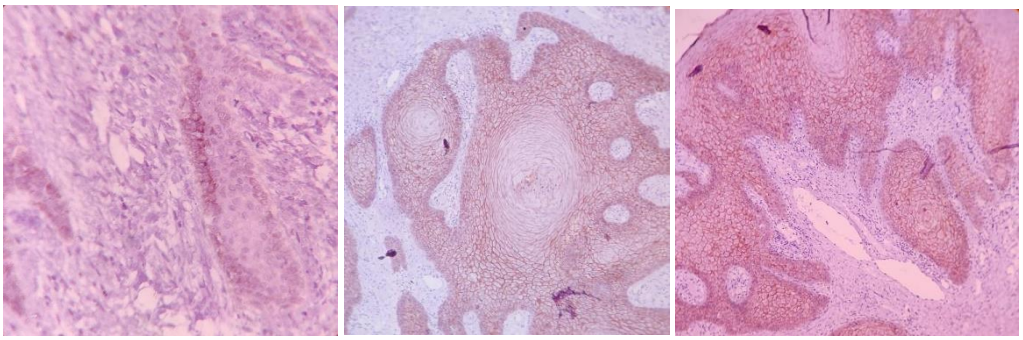
Variables: In the study, sampling was done purely based on different histological grading of Squamous cell carcinoma because it indicates overall prognostic value of the lesion.

III. Results:

There were total 21 tissue samples (n= 21) stained histologically and immunohistochemically in the phase- I of the study to evaluate the SOX2 concentration in different grades of SCCs. The overall time frame of the study was from 2019 to 2023, approximately 4 years. All the samples of the patients were between the age of 30 to 70 years. Total 7 tissue samples of three different histological grading were taken for the analysis. 7 samples from well differentiated squamous cell carcinoma and 7 from moderately differentiated type and 7 from poorly differentiated respectively. A Chi square test performed to evaluate the expression of SOX2 in different histological grades of OSCCs. A statically significant difference was found between histologically grades of OSCCs. Nuclear expression of SOX2 was more prominent in Poor type (85.7%) of OSCC than the Well differentiated type (14.3%) and Moderately differentiated type (42.9%) respectively. **(Table 1, Graph 1)**



(a) **(b)** **(c)**



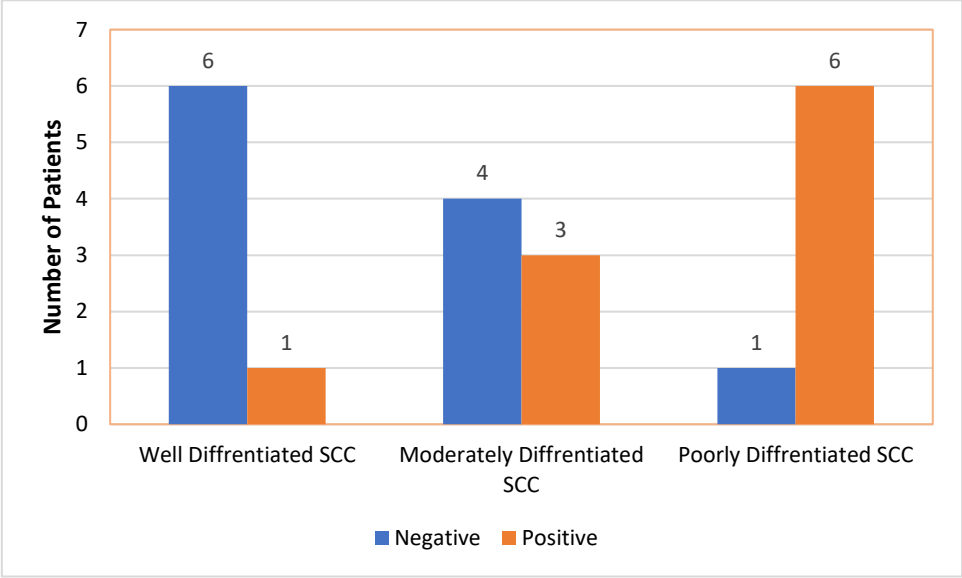
(d) (e) (f)

Figure 1: Histopathological grading of oral squamous cell carcinoma, showing well-differentiated (a), moderately-differentiated (b), and poorly-differentiated (c) cases. IHC section shows proliferation of SOX2 marker in histologically diagnosed OSCC in different grades well-differentiated (d), moderately-differentiated (e), and poorly-differentiated (f) respectively.

Table 1: Comparison of SOX2 levels in different Histological grades of Oral Squamous Cell Carcinoma

Histological Grade of SCC	Negative N (%)	Posistive N (%)	Total N (%)	Chi-Square value Df (Degree of freedom)	P value
Well Differentiated Type	06 (85.7%)	01 (14.3%)	07 (100%)	7.25 Df-2	**0.027 (p<0.05)**
Moderately Differentiated Type	04 (57.1%)	03 (42.9%)	07 (100%)		
Poorly Differentiated Type	01 (14.3%)	06 (85.7%)	07 (100%)		**Significant**

Graph 1:



Statistical Analysis:

The data obtained was subjected to statistical analysis with the consult of a statistician.

The data so obtained was compiled systematically. A master table was prepared and the total data was subdivided and distributed meaningfully and presented as individual tables along with graphs. Statistical procedures were carried out in 2 steps:

1. Data compilation and presentation
2. Statistical analysis

Statistical analysis was done using Statistical Package of Social Science (SPSS Version 19.0; Chicago Inc., USA). Data comparison was done by applying specific statistical tests to find out the statistical significance of the comparisons. Chi square test was applied to evaluate significant differences with respect to SOX2 amongst different histological grading of Oral Squamous cell carcinoma. Significance level was fixed at $P \leq 0.05$.

IV. Discussion:

Cancer stem cells (CSCs) are defined as a small subpopulation of cells in the tumors that possess the ability to initiate neoplasms and sustain tumor self-renewal.[9] Sox2 gene mapping at 3q26 is frequently amplified in OSCC and other cancers. It has been established as an important CSC marker and a key molecule in the development of tumorigenesis in several cancers and thus it is proposed as oncogene.[10] the cancer cells which escape chemo and radiation therapies were supposed to be responsible for the recurrence of tumors. These cells were found to have the ability to exclude anti-tumour drugs and showed resistance to advanced therapies.[11] Arnold et al. reported that epithelial adult stem cells expressing SOX2 may be residual stem niches that originate from embryonic SOX2-positive tissue progenitors. Cai et al. investigated the roles of OCT4 and SOX2 in the reprogramming of oral cancer stem cells. They also stated that oral carcinogenesis may derive from OCT4+SOX2+ reprogrammed stem cells, in which SOX2 plays a major role in the regulation of the CSC niche. Accordingly, it has been proposed that, in the absence of SOX2 expression, CSC self-renewal that sustains tumour growth could be abrogated, therefore, supporting SOX2 inhibition as a potentially relevant therapeutic target for oral cancer.

V. Conclusion:

In many types of human cancer, SOX2 is dysregulated due to gene amplification and protein overexpression. SOX2 overexpression is associated with poor survival of cancer patients. In a way it can be used as a useful prognostic marker for early prediction and better treatment outcomes.

Conflicts of Interest:
None declared

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