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Cancer stem cell niche in oral squamous cell carcinoma and its surgical margins: an immunohistochemistry study

Gargi Sarode[1,*], Anjali Ganjare[2], Roopa Yadahalli[1], Namrata Sengupta[1], Urmi Ghone[1], Vaibhav Ladke[3], Vini Mehta[4], Sachin C Sarode[1]

[1] Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India.
[2] Private Dental Practitioner, Aurangabad, India.
[3] Interdiciplinary Research – CRF, Dr. D.Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India.
[4] Department of Public Health Dentistry, Dr. D.Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India.

*Corresponding author: Dr. Gargi Sarode, BDS, MDS, Ph.D.
Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Sant-Tukaram Nagar, Pimpri, Pune, India.
E-mail: : gargi14@gmail.com
Ph: +91 9823871462
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Abstract

Introduction: With almost 350,000 cancer deaths each year in developed countries, head and neck cancer (HNC) the sixth most frequently noted malignancy [1]. Oral malignancies account for the vast majority of HNC (85%), with oral squamous cell carcinoma (OSCC) being the most common kind. **Objective:** Oral squamous cell carcinoma (OSCC) has a lower survival range and poor prognosis despite the existing advanced treatment modalities due to the formation of metastasis and 'drug resistance' of the tumor cells. The objective of this research was to analyze the cancer stem cell (CSC) niche in lesional tissue of OSCC and it's all surgical margins with the help of CD44, CD133, OCT-4 markers through immunohistochemistry (IHC) study. **Methods:** A total of 11 excisional biopsy tissues of OSCC with four surgical margins (anterior, posterior, lateral and medial) each were selected. The immunoreactivity of CSCs in lesional tissue and surgical margins of OSCC was evaluated with the help of CD133, CD44 and Oct-4 markers. The immunoreactivity was then compared between the lesional tissue and surgical margins, and the outcome were related with patient survival of patient over a time of 3 years. **Results:** CD44 and CD133 in most of the cases showed moderately positive in higher grade OSCC, the weak expression of OCT4 was also expressed in higher grade tumors. **Conclusion:** The increased expression of CD133 and CD44 in advanced grades would be the important result for OSCC cases could suggesting the characterization of CD133 and CD44 in oral mucosal carcinogenesis oral mucosa.

Keywords: Cancer stem cell. Immunohistochemistry. Oral squamous cell carcinoma. Surgical margin.

Introduction

With almost 350,000 cancer deaths each year in developed countries, head and neck cancer (HNC) the sixth most frequently noted malignancy [1]. Oral malignancies account for the vast majority of HNC (85%), with oral squamous cell carcinoma (OSCC) being the most common kind. Despite substantial breakthroughs in modern medicine, oral cancer patients' life expectancy rates hasve not noticeably proved improvementd from the last 20 years [2,3]. After diagnosis, the five-year survival rate of approximately five-year remains at 15–50% [4]. Surgical excision and/or combined chemotherapy and radioation therapy are currently used to treat HNC which can be unpleasant, painful, and disfiguring, consequent in a significant diminution in quality of life [5,6]. Despite undisputable progress in understanding and treatment of OSCC, it remains a significant medical problem.

The cancer stem cell (CSC) hypothesis is based on the initiation, progressiveness, and recurrency of cancer. have led to the cancer stem cell (CSC) hypothesis. It posits a hierarchic framework in tumour genesis and advancement, implying that merely a small subset of independent malignant cells has the power in maintaing the tumour [7]. In numerous forms of malignancies, including HNC, supposed cancer stem cells (CSCs) have been found, isolated, and described with its necessitate for diagnosticis, prognosticis and treatment purpose [3].

In the present study we hypothesize that in OSCC, surgical margins govern the niche of stem cells which renders the patient at risk of recurrence. With the help of immunochemistry (IHC) markers (CD24, CD133, OCT4) we would like to assess the presence of CSCs at the margins and to correlate them with the tumor mass proper.

The CSC hypothesis

According to defination for CSCs they are defined as a known as small subpopulation of cells of cancer cells that constituterepresent a poolassociation of independent cells with the sole quality to origin the disparate line of cancer cells that constitute the tumour [7,8]. CSCs show influential initiation of tumor, demonstrate self-renewal in vivo and possess differentiation capacity [8]. CSCs are immune towards conventional treatment techniques, in adding-on to the self-renewing, differentiative, and regenerative characters [9]. CSCs have the ability to undergo epithelial-mesenchymal transformation (EMT), a crucial stage in healing of wound and embryogenesis [10].

CSC biomarkers used

1. CD 44

One of the most well-known markers for CSCs is CD44, a major cell surface glycoprotein involved in cell adhesion and migration [11]. CD44 can bind growth factors and the metalloproteinases MMP9 through interactions with hyaluronic acid (HA), chondroitin sulphate, and heparan sulphate, inhibiting apoptosis, collagen breakdown, invasion, and neovascularization [12,13]. CD44+ cell frequency has recently been linked to a poor prognosis, more aggressive tumours, and greater rates of recurrence after radiation. CD44 expression has also been proposed as a prognostic indicator, with multiple studies indicating a statistically significant link between CD44 expression and lower 5-year survival [14].

2. CD133

In brain, prostate, lung, skin, liver, and colorectal malignancies, this pentaspan transmembrane glycoprotein has been discovered as a possible CSC marker [15].

Recent research has discovered a link between CD133 expression and carcinogenesis stage, with stage III and IV tumours having higher levels than stages I and II. CD133+ cells obtained from head and neck squamous cell carcinoma cell lines have been shown to have higher clonogenicity, an EMT phenotype, tumour sphere formation, self-renewal, proliferation, multilinear differentiation, and tumorigenicity, according to a number of investigations [16].

3. Octamer-binding transcription factor 4 (OCT4)

OCT4 has been associated with oncogenic processes and plays a vital role in the formation and self-renewal of embryonic stem cells [17,18]. Oct-4 and Nanog may have a role in tumour transformation, tumorgenicity, and metastasis, according to Chiou et al. [17], who discovered elevated expression of these genes in a CSC-enriched subpopulation formed from OSCC sphere forming colonies.

Materials and Methods

A total of 11 excisional biopsy tissues of OSCC with four surgical margins (anterior, posterior, lateral and medial) each (165 total) were selected for the study based on the inclusion and exclusion criteria (Table 1).

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Histopathologically proven OSCC tissue.	Surgical margin not less than 2mm in distance.
Histopathologically proven surgical margins in various grades of OSCC.	

Source: Own authorship.

Immunohistochemistry (IHC) Technique - 3-µm sections were mounted on salinized slides and were used for immunochemical staining. Briefly, sections were de-paraffinized in a series of xylene for 15 min and rehydrated in graded ethanol solutions. Endogenous peroxidase activity was blocked by incubating the sections in 0.3% H_2O_2 in methanol for 30 min. Antigen retrieval was achieved by heat treatment using 10 mM citrate buffer solution DH 6.0 (CD133, CD44, OCT-4). After treatment with normal serum, the sections with primary antibodies were incubated at 4°C overnight. The tagging of the primary antibody was achieved by subsequent application of anti-goat/mouse IgG and avidin-biotin complexes. The reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Myer's hematoxylin and mounted. The membranous expression of CD 133, CD44 and OCT4 was estimated by immunopositivity in lesional tumor tissue and mainly in the tumor closed surgical margins (anterior, posterior, lateral and medial). The

average number of stained cells in 5 visual fields was regarded as the percent ratio of positively stained cells in each section. Staining with these antibodies was considered positive if >10% of tumor cells were stained because a 10% cut-off level has been used in several previous studies. Patients undergoing IHC study were followed for 3 years and later on assessed for the recurrence of surgically treated patients and the survival status was noted.

Statistical Analysis

The obtained information was analyzed by the Statistical Package for Social Sciences (SPSS) for Windows, Version 28.0. (Armonk, NY: IBM Corp) Continuous variables were given in Mean ± Std. Deviation form.

Results

Demographic data and clinicopathological prognosticators

Out of the 11 cases in the study class, 6 male cases and 5 female cases with a mean age of 61.27±10.32 years. Habits of tobacco chewing were found in the patients. (Table 2) The IHC staining intensity of Oct-5, CD44 and CD133 in lesional and all the margins are shown in Figures 6,7, and 8.

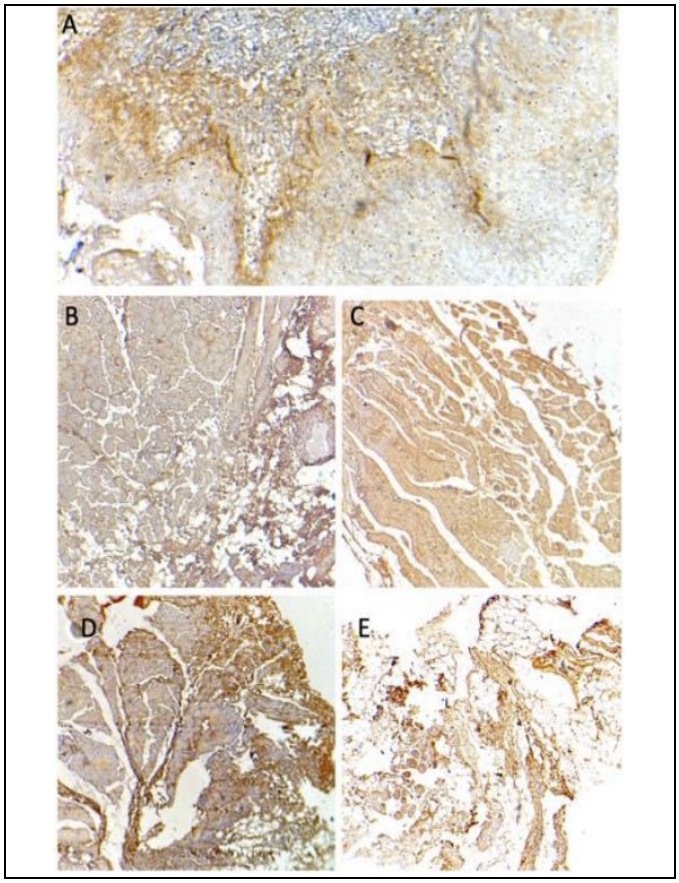

Figure 2. IHC expression of CD44 in 10X power showing moderately expressed a.lesional b. anterior c. posterior d. medial e. lateral.

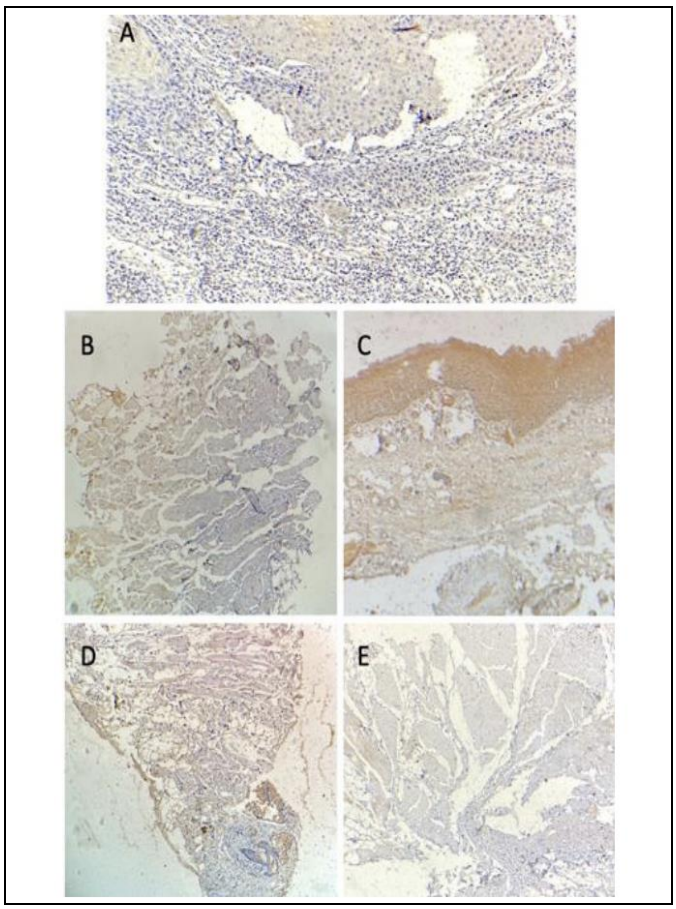

Figure 1. IHC expression of Oct4 in 10X power showing weak expression a.lesional b. anterior c. posterior d. medial e. lateral.

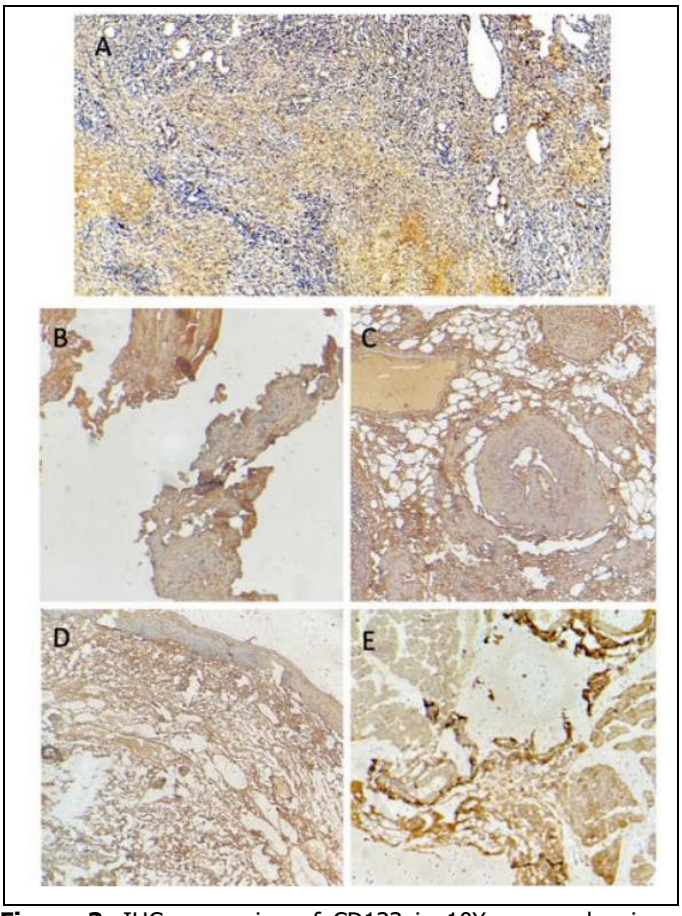

Figure 3. IHC expression of CD133 in 10X power showing moderately expressed A. lesional; B. anterior; C. posterior; D. medial; E. lateral. Source: Own authorship.

Table 2. Demographics and clinicopathological prognosticators.

Sociodemographic data and clinicopathological prognosticators

Variables	Study group n (%)
Total number of cases	n = 11
Sociodemographic data	
Age (years)	
Mean ± standard deviation, range	
	61.27 ± 10.32, 47-80
≤61.27 years	
>61.27 years	6 (54.5)
	5 (45.4)
Gender	
Male	
Female	6 (54.5)
	5 (45.4)
Clinicopathological prognosticators	
Habits :	3 (27.27)
Tobacco	1 (9.09)
Mishri	1 (9.09)
Paan,	3 (27.27)
Gutka	3 (27.27)
No habit	
Tumor differentiation:	5 (45.4)
Well differentiated	4 (36.36)
Well- moderately differentiated	1 (9.09)
Moderately differentiated	1 (9.09)
Poorly differentiated	
Metastasis to neck	4 (36.36)
Yes	7 (63.63)
No	
Patient survival :	5 (45.4)
Not Survived	2(18.18)
Surviving	4 (36.36)
Not recorded	

*lip, buccal mucosa, alveolar mucosa, retromolar and gingivobuccal complex

Source: Own authorship.

Assessment of Oct 4, CD 44 and CD 133 in different margins and lesional tissue

Lesional tissue showed 90% weak expression and 10% moderate expression of Oct-4. CD-44 was expressed moderately in 90%, strongly in 9.09% of cases. A total of 90.09% cases showed moderate expression of CD 133 and 9.09% showed strong expression (Figure 4). Anterior margin expressed 100% weak expression of Oct4. CD-44 was expressed weakly in 27.27% cases, moderately in 63.63% cases, 9.09% showed strong expression. CD 133 was expressed moderately in 90.09%, and 9.09% showed strong expression of the marker (Figure 5). Posterior margin showed 90.90% weak and 9.09% moderate expression of Oct 4 marker. 27.27% weak, 54.5% moderate, and 18.18% strong expression of CD44 was seen. All cases showed 90.90% weak and 9.09% moderate expression of CD 133 marker (Figure 6). Medial margin expressed 90.90% weak and 9.09% moderate expression of Oct 4 marker. 27.27% weak, 63.63% moderate and 9.09% strong expression of CD 44 was observed in this margin of all cases. CD133 was expressed moderately in 72.72% cases and strongly in 27.27% cases (Figure 7). In lateral margins all cases showed weak expression of Oct 4 marker. CD 44 was weak in 27.27% cases, moderate in 72.72% cases. CD133 was weak in 9.09% moderate in 72.72% and strong in 18.18% cases (Figure 8).

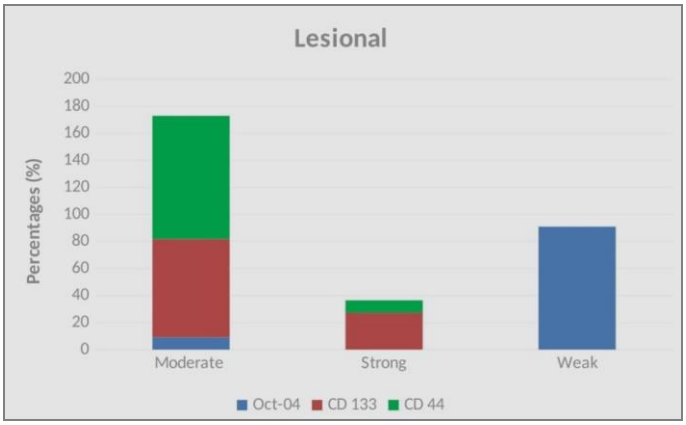

Figure 4. Graphical expression of Oct 4, CD 44 and CD 133 in lesional tissue.

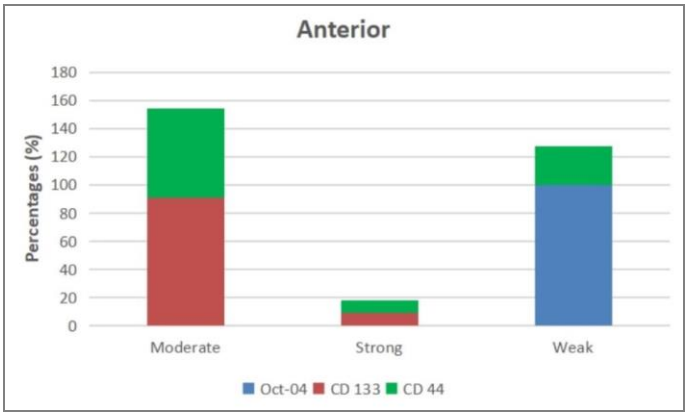

Figure 5. Graphical expression of Oct 4, CD 44 and CD 133 in the anterior margin. Source: Own authorship.

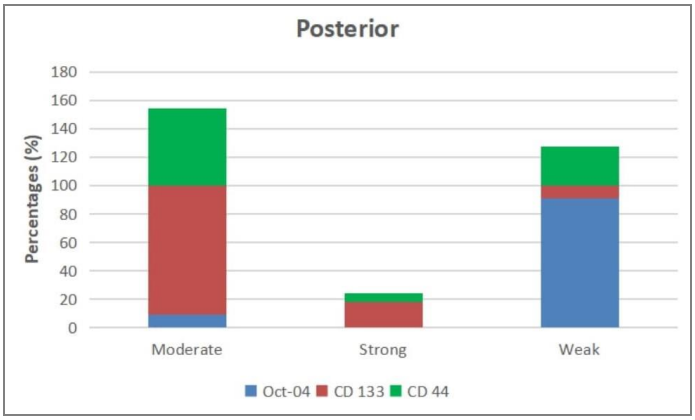

Figure 6. Graphical expression of Oct 4, CD 44 and CD 133 in posterior margin.

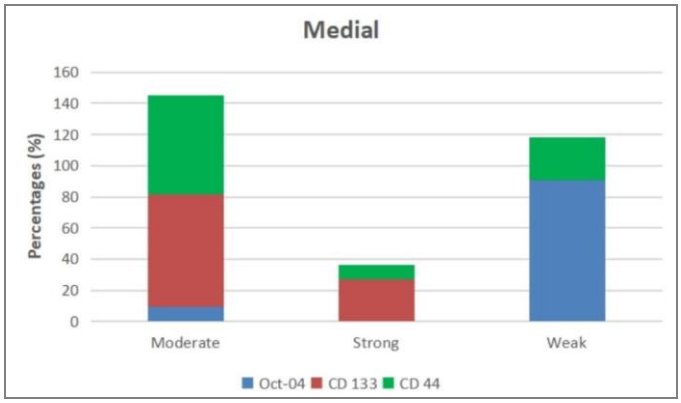

Figure 7. Graphical expression of Oct 4, CD 44 and CD 133 in medial margin. Source: Own authorship.

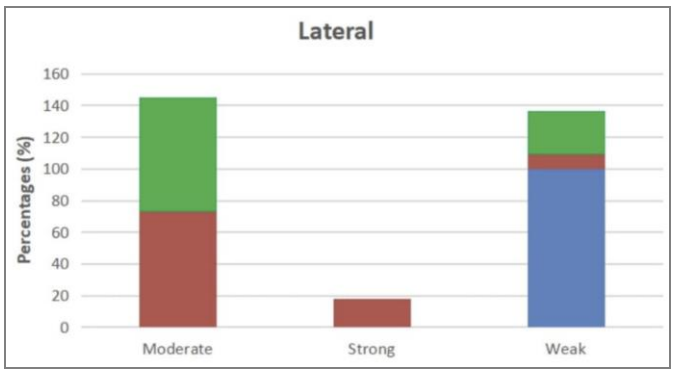

Figure 8. Graphical expression of Oct 4, CD 44 and CD 133 in lateral margin. Source: Own authorship.

Discussion

The prevalence of OSCC is rising worldwide, special in Asian nations, and is partially attributable to the activity of habits related to high risk such as smoking, drinking alcohol, and chewing betelnut. Along with radiotherapy and chemotherapy, surgery is the chosen course of treatment. However, OSCC treatment is still difficult because of the aggressive nature of the disease, which causes relapses. The most important stage in treating OSCC is surgical resection, which also involves removing any normal tissue to evaluate the success of the operation. Even so, up to 30% of individuals with histologically negative surgical margins diagnosed during surgery will experience recurrences [19].

It is most likely a result of the tumour cells that are still present but undetectable following regular histological examination in the negative margins (minimal residual cancer) [20]. Another hypothesis, known as the "theory of field cancerization," postulates the existence of abnormal alteration of the tissues around the tumour and links their persistency to the emergence of locally recurring or secondary tumours [21].

Both ideas are close incidental to an inadequate assessment histologically of the tissues that appear to be normal and surround the tumour, indicating the need to investigate additional techniques for a higher degree of accuracy in the assessment of surgical margins. It is critical to identify lesions that are very susceptible to developing into OSCC early on. This can be accomplished by locating CSCs that are linked to the emergence and spread of cancer. These CSCs are created as a result of the accumulation of mutations in healthy stem cells, and they may be recognised by a number of particular cell surface antigens called CSC markers.

Even though the theory of CSC has been justified for various malignancy, detailed investigation is necessary to look into the function of CSCs in the developing and advancement of OSCC. Earlier studies disclosed about the reflection of these markers may enhance in normal cells close to neoplastic and pre-neoplastic tissues, that indicates molecular level early modification in normal cells adjacent to malignant cells and the affirmable role of CSCs in the cognition of carcinogenesis [12]. Oral cancer-derived CSCs has a significance in growth of tumour and metastasis. They also exhibit the ability to withstand radiation and chemotherapy [22]. It is crucial to understand the therapeutic function of CSCs in OSCC as well as the mechanisms behind their activation.

CD44 is one among the most often determined marker of cancer stem cell in tumors of solid variant, which is disclosed to be by the Wnt pathway [23]. Several publications discovered that an elevated levels of transcriptional CD44 suggested disease progression, although several of these studies demonstrated a negative connection with CD44 levels and prognostic value in patients with oral cancer. In addition, Lin et al. [24] found that cases of local progressive oral or oropharynx cancer had more levels of CD44 mRNA in their peripheral blood than healthy individuals, and that a high level of CD44 mRNA was importantly associated with a bad prognosis. Rajarajan et al. [25] established about level of mRNA in CD44 was increased significantly in OSCC. In the present study, the lesional tissue and other margins showed moderate expression of CD44. Already the positive correlation between the expression of CD44 is seen in other studies and poor prognosis of the patient in malignancy which can be correlated with the current study.

In regards with parameters related to survival in cases of OSCC, less number of studies has been performed on markers associated. Epithelial cells and stem cells of somatic in several tissues have been discovered to express the CSC marker CD133 (Prominin-1) [26]. According to Wu et al. (2009) [27], CD133+ cells displayed an EMT phenotype, self-renewal, differentiation, proliferation, and tumorogenicity in OSCC. Its function is unclear, although high expression has been linked to a ill

prognostication in case of esophageal malignancy and has been proven to enhance chemoresistance and tumour recurrences [28]. In the present study, Cd133 is mostly expressed moderately in all the tissues examined and this marker is linked with the poor prognosis, resistance to chemotherapy and recurrence of tumor.

OCT4 in most of the cases is weakly expressed in the current study. Oct-4 is known for functioning in transformation of tumour, tumorgenicity, and metastasis. And even elevated aspect of these genes in a CSC ameliorated population formed from OSCC is noted [17]. Carcinogenicity mediated by Oct4 is related by regulating the EMT [29]. In relation with the histopathological grading of OSCC with OCT4 expression it is noted that the higher grade showed higher expression [30]. Most of our cases fall in the category of well or well to moderate differentiated OSCC which goes with weak expression as they fall in low grade.

The recent seen of survival is getting worst with the 5 years survival rates which is descending to 50%, with continual treatment failures and frequent cases of relapse/recurrence [31]. In the present study the followup of 3 years has been done for survival and non-survived cases out of 11 were 5 (45.4%) and 2(18.18%) were found to be survived and follow up was lost for 4 (36.36%) cases. In case of not survived patients all of them showed recurrence approximately after 1.5 to 2 yrs. And the expression of CSC markers in the tissue of all the non-survived cases showed, weak expression for OCT 4, moderate expression for CD44 and CD133. One of the prime explanations for these failures might be the existence of cancer stem cells and even, notable, recent evidence suggesting their immune to treatment with conventional method and are the "thrust" of localized recurrence and metastatic spread [32].

Future studies may be required with more number of sample to explicate the relationship of prediction, carcinogenicity and endurance rate with the manifestation of CSCs markers. The present study markers CD44 and CD133 in most of the cases showed moderately positive in higher grade OSCC, even the weak expression of OCT4 was seen in higher grade tumors. Suggesting that this might symbolize a point of reference molecule for the management of advanced cancer.

Conclusion

It was concluded that the increased expression of CD133 and CD44 in advanced grades would be the important result for OSCC cases could suggesting the characterization of CD133 and CD44 in oral mucosal carcinogenesis oral mucosa.

CRediT

Author contributions: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Supervision; Writing - original draft; Writing-review & editing- All authors.

Acknowledgment

Not applicable.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Funding

Not applicable.

Data Sharing Statement

The datasets generated and analyzed during the current study are not publicly available due to participant privacy and institutional ethical restrictions but are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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