Original Article

Quantitative Analysis Of CD133 & OCT 4 As Prognostic Markers In Oral Squamous Cell Carcinoma

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Abstract

Aim: To evaluate the expression of CD133 and OCT 4 in Oral Squamous Cell Carcinoma.

Materials and methods: The study included a total of 50 samples of Archival tissue blocks that were categorised as control and cases (Oral squamous cell carcinoma) and further as Well-differentiated & Moderately squamous cell carcinoma. The sections of both Group I and group II were subjected to Immunohistochemical staining using CD133 antibody and anti-Oct-4 antibody. The cells were counted and the data obtained were compared for the significance keeping $p<0.05$ as significant.

Results: Statistical significance is observed on the correlation of CD133 and OCT 4 expression when the mean number of positive cells is compared between the control and study groups. A high statistical significance is observed on the correlation of CD133 and OCT 4 expression when the mean number of positive cells is compared in normal mucosa and moderately differentiated Squamous cell carcinoma.

Conclusion: The quantitative expression of CD133 and OCT 4 increased from normal mucosa to the well-differentiated variation of oral squamous cell carcinoma, and subsequently to the moderately differentiated variant, suggesting that CD133 and OCT 4 plays a role in determining the prognosis of oral squamous cell carcinoma patients.

Keywords: CD133, OCT 4, Prognostic Markers, Oral Squamous Cell Carcinoma, Immuno Markers.INTRODUCTION

Introduction

Oral squamous cell carcinoma (OSCC), the sixth most frequent Head & Neck cancer, kills almost 200,000 people annually.1 India reports 100,000 instances annually. Sri Lanka, Bangladesh, and Pakistan reported significant incidence. 2 Due of its high mortality and low treatment rate, OSCC is a global health issue. OSCC is men's most prevalent carcinoma and women's third. 3,4 90%–95% of intraoral tumors are it.5 OSCC in youth has increased from 0.4% to 13%. 6 Due to this perspective, most OSCC instances are either missed or diagnosed late. Late diagnosis causes widespread metastases and short survival. 7 OSCC has a 50% five-year survival rate. 8 OSCC is caused by cigarettes, alcohol, and viruses such Human Papillomavirus, Epstein-Bar virus, and Herpes Simplex Virus-1 causes cancer. 9,2 Complex invasiveness and recurrence are the main causes of therapy failure and poor prognosis. 10

The tumor stage at diagnosis greatly affects prognosis and survival. 11 Clinical, pathological, and biological OSCC prognostic factors are disputed. Like many heterogeneous solid tumors, 12 OSCCs have subpopulations of

"tumor-initiating cells" (TIC), malignant stem cells that drive tumor growth. 13 An emerging idea of cancer stem cells (CSC) suggests that a group of tumor cells self-replicate, carcinogenesis, and recurrence of cancers including head and neck squamous cell tumors. Lack of reliable markers makes detecting cancer stem cells difficult. 4 Surface indicators identify CSCs.

Under research, CD133 is the best surface marker for solid tumor CSC identification. 14, 15 Human CD133 is the mouse prominin-1 homolog. A subpopulation of human fetal liver and bone marrow-derived CD34+ hematopoietic stem and progenitor cells expresses it. Human OSCC and animal models of oral cancer have CD133-expressing normal and malignant stem cells. 14 Highly conserved antigen CD133 (AC133). 16 CD133 expression predicts tumor recurrence, malignant progression, and stages in leukemia, brain tumors, retinoblastoma, renal tumors, pancreatic tumors, colon carcinoma, prostate carcinoma, hepatocellular carcinoma, thyroid carcinoma, melanoma, and oral cancer. 17 It marks hematopoietic stem cells, endothelial progenitors, and angiogenesis. 18,19 CD133 also isolates tumor-inducing colon and glioma cancer stem cells. 20,21 CD133 is important for stem cell classification and isolation, tumor biology, growth, and development. Oral squamous cell carcinoma CD133 cells showed self-renewal, differentiation, proliferation, tumorigenicity, clonogenicity, and EMT. 4 Several studies found that CD133 expression in cancer cells resists therapy, indicating that they may influence OSCC's clinical fate. 22

Oct-4 is a human octamer-binding transcription factor protein encoded by the POU5F1 gene. 2,23 Oct-4 affects carcinogenesis, transformation, and prognosis. 8 Pluripotent embryonic stem and germ cells express it. Oct-4 is down-regulated during differentiation, demonstrating its importance in mammalian development. POU transcription factors are DNA-binding proteins that activate genes with cis-acting elements containing the octamer motif in their promoter or enhancer regions. Undifferentiated embryonic stem (ES) cells' pluripotency and self-renewal depend on Oct-4. Oct-4 can transform human somatic fibroblasts into inducible pluripotent stem cells that resemble embryonic stem cells (iPSC). Cancers such germ cell tumors, breast, cervix, oral, prostate, lung, stomach, brain, liver, and ovarian overexpress Oct-4. Oct-4 also affects CSCs. 2,23 POU genes regulate Oct-4 and stem cell self-renewal. Only pluripotent stem cells express Oct-4. It is germ cell tumor CSC marker. Oct-4 expression is linked to CSC behavior, tumorigenic potential, and aggressive clinical characteristics such metastasis and disease progression.

In the current study the OSCC samples' CD133 and Oct-4 expression by immunohistochemistry (IHC) to determine their role in OSCC prognosis is evaluated.

MATERIALS AND METHODS

Study description:

The present study was carried out by retrieving archived records, Archival tissue blocks of histopathologically diagnosed oral squamous cell carcinoma cases and normal oral mucosa tissues from the Department of Oral and Maxillofacial Pathology and Oral Microbiology.

The study included a total of 50 samples of Archival tissue blocks and they are categorized into two groups as follows:

- Group I: Normal mucosa (25) – Control group
- Group II: Oral squamous cell carcinoma (25) – Study group

Group II consists of the well and moderately differentiated squamous cell carcinoma variants. To compare grade-wise, these variants are considered as

- Group II(a) - Well-differentiated squamous cell carcinoma
- Group II(b) - Moderately differentiated squamous cell carcinoma

All the samples were subjected to routine hematoxylin and eosin to reconfirm the diagnosis histologically and then immunohistochemical staining for the CD133 marker. The number of immunopositive cells was assessed in each sample, and the score was given accordingly.

Methodology

Serial sections of 4 microns were obtained from the Archival tissue blocks. The sections of both groups I and group II were first subjected to routine hematoxylin and eosin stain for examination under the microscope to reconfirm the diagnosis histopathologically. Later other sections of both Group I and group II were subjected to Immunohistochemical staining using CD133 antibody and anti-Oct-4 antibody.

Interpretation

Positive CD133 expression was seen as a light brown stain in the cytoplasm under Olympus BX51 Penta Head Microscope made in Japan. All light brown stained areas demonstrating positivity for CD133 were identified and captured at a magnification of 20X on ten representative areas with a minimum of 100 cells per field using the Jenoptik charge-coupled device made in Germany. The cells showing positivity for CD133 were captured on ten usual areas with a minimum of 100 cells per field. The captured images were displayed, and positive cells are counted by using the grid tool in micaps-micro view 3.7 version software to avoid repeated count of the cells. Fig 1,2

Statistical Analysis:

The collected data was noted in the excel sheet, and statistical analysis was carried out using software, Statistical Package for Social Sciences (SPSS) version 20.0. The normal distribution of the number of positive cells was calculated by one-way ANOVA, Independent ‘t’ test, and Tukey's post hoc test.

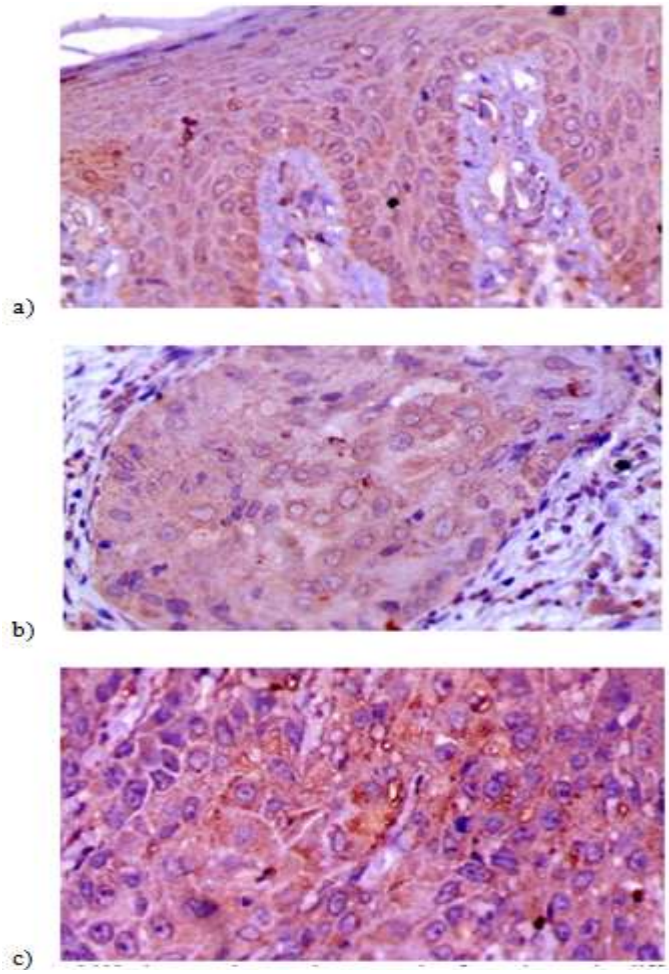


Figure 1: Photomicrograph showing 20X view of CD133 stained a) normal oral mucosa; b) Well Differentiated Squamous Cell Carcinoma; c) Moderately Differentiated Squamous Cell Carcinoma.

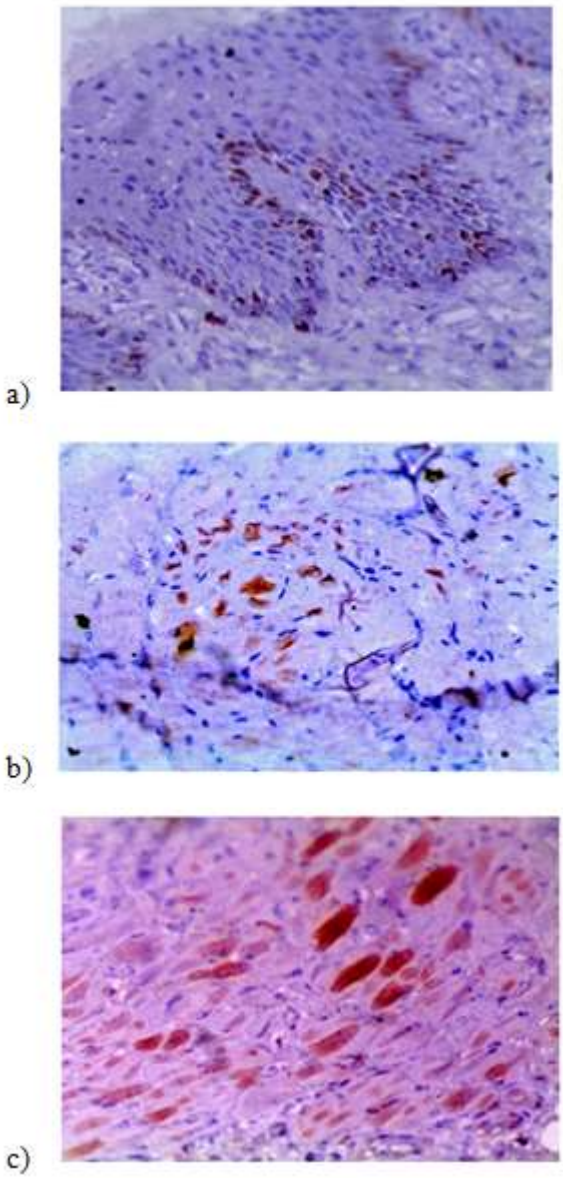


Figure 2: Photomicrograph showing 20X view of Oct-4 stained a) normal oral mucosa; b) Well Differentiated Squamous Cell Carcinoma; c) Moderately Differentiated Squamous Cell Carcinoma.

RESULTS

1. Assessment of CD133

a) Number of positive cells

Assessment of the mean number of positive cells between Group I and Group II reveals that the mean value of the number of positive cells in Group I was 82.28 ±43.47, and for Group II, the mean value is 153.16 a ± 85.82 and it showed a high statistically significant difference between Group I and Group II ($p < 0.0006$). Evaluation of the mean number of positive cells in the Group - I, II(a), II(b), were 82.28±43.47,120.38, ±64.96,188.66, ±93.92, respectively. The statistical analysis revealed that the mean number of positive cells was highly significant in Group II(b), followed by Group II(a) ($p < 0.0001$). (Table 1)

b) Gender-specific mean positive cell count

The statistical analysis for Gender wise difference in the mean number of positive cells I, II(a), II(b) was done by an independent sample t-test. It showed a more significant number of positive cells - 88.5±53.56 in female samples compared to male samples74.36±26.14. In group II(a), male samples presented a mean number of positive cells - 132.11±69.45 compared to female samples in group II(a), and the group II(b) male samples showed a more significant number of positive cells - 197.7±100.88 compared to females - 143.5 ± 23.3. But there are no significant differences found between males and females among the three groups. (Graph 1) It revealed that both males (197.7±100.88) and females (143.5±23.3) expressed the mean number of positive cells highly in Group II(b). It is informed that the presence of highly significant differences among the three groups ($p < 0.002$), but no significant differences were found among females ($p > 0.098$). (Graph 2)

c) Pairwise comparisons

Multiple pairwise comparisons of the mean number of positive cells were made by using Tukeys post hoc test among three groups. It revealed that the existence of statistically significant differences between Group I and Group II(b) ($p < 0.0001$), between Group I and Group - II(a) ($p < 0.037$), and between-Group II(a) and Group II(b) ($p < 0.044$). (Table 2)

Table 1: Assessment of the mean number of positive cells between Group I and Group II and subcategories.

Group	n	Mean (SD)	P-value
Group I	25	82.28 ±43.47	**0.0006***
Group II	25	153.16 ± 85.82	
Subcategories			
Group I	25	82.28 ± 43.47	**0.0001***
Group II(a)	13	120.38 ± 64.96	
Group II(b)	12	188.66 ± 93.92	

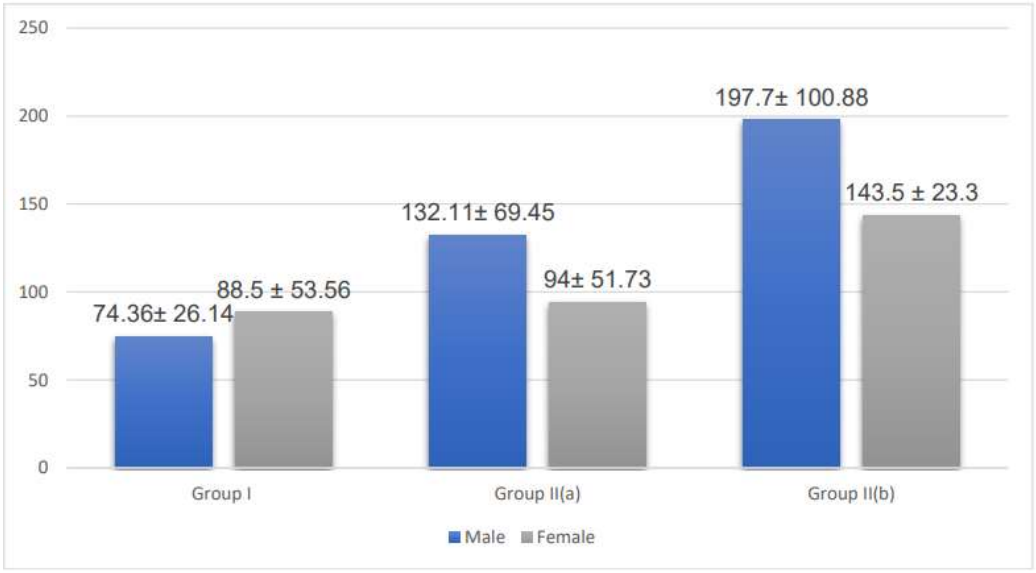
*p≤0.05

Table 2: Multiple pairwise comparisons of the mean number of positive cells

Group	n	Mean (SD)	P-value
Group I Group II(a)	25	82.28 ± 43.37	**0.037***
	13	120.38 ± 64.96	
Group I Group II(b)	25	82.28 ± 43.37	**0.0001***
	12	188.66 ± 93.92	
Group II(a) Group II(b)	13	120.38 ± 64.96	**0.044***
	12	188.66 ± 93.92	

*p≤0.05

Graph 1: Gender wise differences in the mean number of positive cells in the three study groups



Graph 2: Group-wise differences in the mean number of positive cells stratified by gender

2. Assessment of Oct-4

a) Number of positive cells

Assessment of the mean number of positive cells between Group I and Group II reveals that the mean value of the number of positive cells in group I was 72.32 ± 34.3, and for group II the mean value was 101.44±40.8 and it showed a statistically significant difference between group I and group II with a p-value is 0.008. Evaluation of the mean number of positive cells in the group - I, II (a), II (b), were 72.32±34.3, 96.69±38.8, 106.58±44.0, respectively. The statistical analysis revealed that the difference in the mean number of positive cells was highly significant in group II (b), followed by group II(a) with a p-value of 0.027. (Table 3)

b) Gender-specific mean positive cell count

The statistical analysis for Gender wise difference in the mean number of positive cells among the group - I, II (a), II (b) was done by an independent sample t-test. The group I showed more positive cells- 81.07 ± 30.1 in female samples, compared to males samples 61.18 ±30.1. In group II (a), male samples presented a more mean number of positive cells103 ± 44.0 than female samples 94.5 ±14.5. In group II (b), female samples showed more positive cells 123±24.0 than males 103.3 ±47.3. But there are no statistically significant differences found between males and females among the three groups with p values 0.115, 0.89, 0.58, respectively. (Graph 3)

The group-wise difference in the mean number of positive cells was stratified by gender and was statistically analyzed using a one-way analysis of variance (ANOVA). It reveals that Males expressed an almost equal mean number of positive cells in group II (a) (103 ± 44.0) and group II (b) (103.3 ±47.3). Females expressed a high mean number of positive cells in group II (b) (123± 24.0) than group II (a) (94.5 ± 14.5). The above data indicated significant differences among the three groups within males P-value 0.037 and females with a P- value of 0.045. (Graph 4)

c) Pairwise comparisons

Multiple pair wise comparisons of the mean number of positive cells were made using Tukey's post hoc test among three groups. It revealed statistically significant differences between group I and group II (b) with a p-value of 0.013. (Table 4)

Table - 3: Assessment of the mean number of positive cells between Group I and Group II

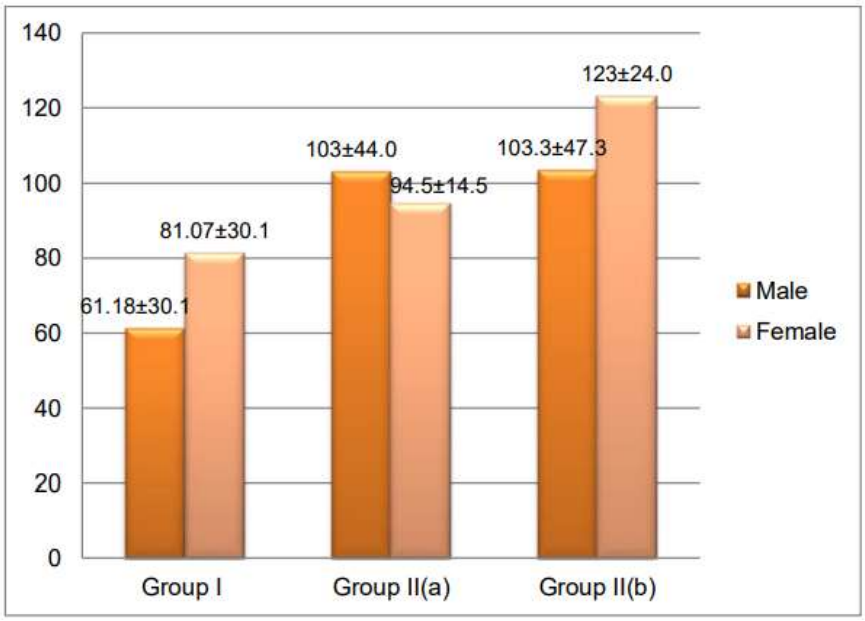
Group	**n**	**Mean (SD)**	**P-value**
Group I	25	72.32 ± 34.3	0.008*
Group II	25	101.44± 40.8	
Subcategories			
Group I	25	72.32 ±34.3	0.027*
Group II(a)	13	96.69 ±38.8	
Group II(b)	12	106.58 ±44.0	

Table - 4: Multiple pair wise comparisons of the mean number of positive cells

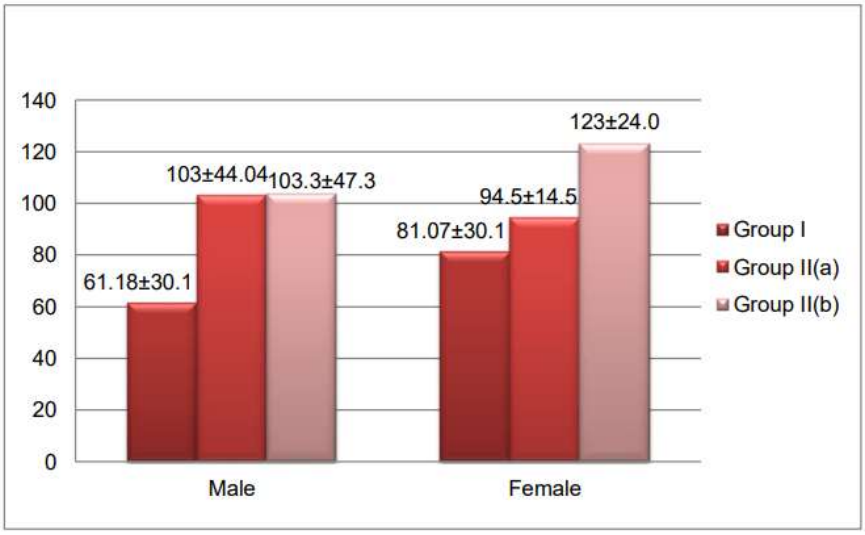
Group	**n**	**Mean (SD)**	**P-value**
Group I Group II(a)	25	72.32 ±34.3	0.054
	13	96.69 ±38.8	
Group I	25	72.32 ±34.3	0.013*
Group II(b)	12	106.58 ±44.0	
Group II(a)	13	96.69 ±38.8	0.557
Group II(b)	12	106.58 ±44.0	

p≤0.05 *

Graph- 3: Gender wise differences in the mean number of positive cells in the three study groups



Graph -4: Group-wise differences in the mean number of positive cells stratified by gender



DISCUSSION

When it comes to tumor growth, metastasis, and the ability to withstand chemotherapy and radiotherapy, cancer stem cells from oral cancer play a crucial role. The identification of the CSCs' therapeutic role in OSCC as well as their activation pathways is helped by CD133. The prostate, kidney, liver, skin, lung, colorectal, and neural tissues all contain somatic stem cells and epithelial cells that express CD133 (prominin-1). The EMT phenotype, self-renewal, differentiation, proliferation, tumorigenicity, and CD133 positive cells were all present in oral squamous cell carcinoma. Cell cytoplasm is where it expresses itself.15,16

In comparison to the healthy oral mucosa, this study found that oral squamous cell cancer expressed more mean CD133-positive cells. The association between oral squamous cell carcinoma variants that have undergone moderate differentiation and those that have undergone well differentiation statistical significance is apparent. As oral squamous cell carcinoma progressed from normal mucosa to a well-differentiated variant and finally to a moderately-differentiated variant, the quantitative expression of CD133 increased, suggesting that CD133 may play a role in determining the prognosis of patients with oral squamous cell carcinoma. In 2017, JiaJia Qi et al. looked into how CD44 and CD133 are expressed in healthy mucosa, oral diseases that may be cancerous, and oral

squamous cell carcinoma. They noticed that CD133 was expressed in oral squamous cell carcinoma, normal oral mucosa, and other conditions that could be cancerous. 24 The expression of CD133 in oral squamous cell carcinoma subtypes, including superficial oral squamous cell carcinoma, conventional and basaloid variations of SCC, was examined by De oliveira J et al. in 2017 and immunohistochemical staining was used. In superficial squamous cell carcinoma, they found that CD133+ expression was statistically exceptional. 25 Singh A et al. examined the immunohistochemistry (IHC) expression of CD133 and OCT-4 in OSSC samples and linked it with several clinicopathological factors in 2018. Between stages I and II, 20.6% of the samples showed positive CD133 staining, while between stages III and IV, 79.4% of the samples did. 11

In the current study, it was found that OSCC exhibits greater mean numbers of Oct-4-positive cells than normal mucosa, with the mean numbers of positive cells in the study group being about 40% more than those in the control group. As a result of the stark disparity, it was clear that group II's Oct-4 expression was more overt. This suggests that Oct-4 is essential for predicting the prognosis of OSCC. All of the dysplastic cells in the superficial epithelium of OSCC, according to Vijayakumar G et al., observation in 2019, were positive for the Oct-4. 26

It was discovered in the current investigation that group I's basal layer exhibited a significant amount of the maximum mean number of positive cells. Due to the detached cells' proliferation into the connective tissue, group II's expression was not restricted to the basal layer. The above-mentioned studies and current research are corresponding. Oct-4, SOX2, and NANOG expression were examined by Fu TY et al. in 2016 in tumor tissues, normal tissues around tumors, and normal oral tissues. According to their findings, only the cell nucleus expressed oct-4. 27 In line with the other investigations, the current study demonstrated that Oct-4 is expressed in the nucleus.

CONCLUSION

Current study's results suggest that CD133 and OCT 4 expression in oral squamous cell carcinoma was significantly increasing from well to moderately differentiated squamous cell carcinoma. CD133 and OCT 4 play a role in assessing the rate of malignant transformation based on its quantitative expression. They may also act as a prognostic marker in evaluating the prognosis of squamous cell carcinoma. Further studies are suggested to corroborate the findings of this study.

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