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A study of the expression of CD44 and Bcl-2 in oral squamous cell carcinoma and it's correlation with histological grading

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***Abstract*---**Introduction :Oral squamous cell carcinoma is one of the most prevalent cancers worldwide, with a global incidence of around 3,54,864 new cases and 1,77,384 deaths every year.Aim :To demonstrate and assess the prognostic significance of the expression of CD44 and Bcl-2 in squamous cell carcinomas of oral cavity by it's correlation with the histological grade of the tumour.Methodology :This is a hospital based prospective study done over a period of 3 years at Kakatiya Medical College/MGM Hospital, Warangal, Telangana and includes 76 cases diagnosed as OSCC. Immunohistochemistry was done with markers CD44 and BCL2. ResultS:61.9% of WDSCC cases showed weak expression of CD44, 56.3% of MD SCC cases showed moderate expression, 50% of PD SCC cases showed strong expression and 50% showed moderate expression. 71.5% of WD SCC cases showed weak expression of BCL2, 46.9% of MD SCC cases showed moderate expression, 50% of PD SCC cases showed strong expression and 50% showed moderate expression. Conclusion: The present study, has showed that there is

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significant correlation between the histopathological grade of OSCC and the overexpression of CD44 and Bcl-2.

***Keywords*---**BCl2, CD44, oral squamous cell carcinoma, histological grading.

Introduction

Squamous cell carcinoma is the most frequent malignant tumour of the head and neck region. It accounts for 90% of malignant tumours of the oral cavity. The annual incidence of cancers of oral cavity worldwide is around 3,54,864 cases with around 1,77,384 deaths each year[1]. Male to female ratio ranges from 2:1 to 4:1.It is the most common cancer in India with a large fraction of cases occurring in males in their productive years of life. Males are affected more often than females because of heavier indulgence in both tobacco and alcohol consumption. Patients with tumours of same clinico-pathological stage do not have similar disease progression, response to therapy, rate of disease recurrence and survival. Therefore there is a need to understand the prognostic relationship of OSCC with various molecular markers, potentially offering new methods for early diagnosis and treatment alternatives[2].

The poor prognosis of oral SCCs is related to its high propensity for local invasion and development of nodal metastasis[2]. CD44is a ubiquitous transmembrane cell surface molecule, widely distributed in normal adult and fetal tissues. It is involved in cell - cell interaction, cell adhesion and migration binding withhyaluronin, extracellular matrix proteins and growth factors. It is also involved in cell proliferation, cell differentiation, cell migration, angiogenesis, presentation of cytokines, chemokines and growth factors to corresponding receptors. Cancer cells that undergo anepithelial to mesenchymal transition (EMT) acquire stem cell-like properties andshow an increase in CD44 expression. These cancer cells with an EMTphenotype show increased invasiveness and are more resistant to chemotherapy[3].

BCL2 is an anti apoptotic protein. In normal proliferating epithelium, it is expressed in the stem cell zones like basal layers, where it acts to prevent death of cells in the regenerative compartment. Overexpression of BCL2 results in an alteration of programmed cell death with the persistence of cells that fail to die, favouring the accumulation of new mutations, which can result in the appearance of cells with malignant phenotype[4]. The increased expression of Bcl-2 is essential not only for oral carcinogenesis but also influences the progression of the disease because it increases the survival rate of neoplastic cells, allowing new genetic mutations to accumulate, granting them higher resistance to chemotherapy and radiotherapy[5].

Material and Methods

This is a hospital based prospective study, conducted over a period of 3 years at Kakatiya medical college/MGM Hospital, Warangal, Telangana and includes 76 cases diagnosed as OSCC out of which 42(55%) were well-differentiated, 32 cases

(42%) were of moderate differentiation, and 2 cases (3%) were poorly-differentiated SCCs. All cases diagnosed as OSCC were included and tumours with insufficient viable tumour cells for accurate evaluation were excluded. Tumours were graded according to Broder's criteria into well, moderate and poorly differentiated OSCC. IHC was done with the markers CD44 and BCL2 and immunohistochemical staining was graded as weak, moderate and strong.
Ethics: ethical clearance was obtained from the institution
Analysis:-The data was collected, coded and entered in Microsoft excel. The Chi square test was applied wherever necessary. A P-value of less than 0.05was considered significant.

Table no.1; grading of IHC

Expression score (ES)	Percentage of cells stained
1	1%-25%
2	26%-50%
3	51%-75%
4	>75%
Intensity score (IS)	Intensity of staining
1	Mild
2	Moderate
3	Strong
Total score (TS)	Grading
1-4 points	Weak +
5-8 points	Moderate ++
9-12 points	Moderate ++

Results

In this study, age group ranged from 21 years to 80 years. Of these, the highest incidence of OSCC was seen in the age group of 41-60 years, consisting of 33 cases, and lowest in the age group of 21-40 years, with 12cases. Out of the 76 cases of SCC, 43 cases (57%) were seen on the tongue, 13 in the buccal mucosa, 7on the soft palate, 5 on the lips, 3 cases each on the hard palate and floor of the mouth and 2 cases in the retromolar trigone area. Out of the 76 cases, 42(55%) were well-differentiated, 32 cases (42%) were of moderate differentiation, and 2 cases (3%) were poorly-differentiated SCCs.

Table no 2 : age and gender distribution

VARIABLES	RANGE	FREQUENCY	PERCENTAGE
AGE IN YEARS	21-40	12	15.8%
	41-60	33	43.4%
	61-80	31	40.8%
GENDER	MALE	68	89.5%
	FEMALE	8	10.5%

Table no 3: distributionbased on the site of the tumour

SITE	FREQUENCY	PERCENTAGE
TONGUE	43	57%
BUCCAL MUCOSA	13	17%
SOFT PALATE	7	9%
LIPS	5	6%
HARD PALATE	3	4%
FLOOR OF THE MOUTH	3	4%
RETROMOLAR TRIGONE	2	3%

Table no 4 : distribution based on the grade of the tumour

GRADE OF THE TUMOR	FREQUENCY	PERCENTAGE
WELL DIFFERENTIATED OSCC	42	55%
MODERATELY DIFFERENTIATED OSCC	32	42%
POORLY DIFFERENTIATED OSCC	2	3%

CD44 was expressed as membranous positivity in all the cases of OSCC, with 32 cases (42.1%) showing weak positivity, 29 cases (38.2%) showed moderate grade of positivity and 15 cases (19.7%) showed strong CD44 positivity.61.9% of WDSCC cases showed weak expression of CD44, 56.3% of MD SCC cases showed moderate expression of CD44, 50% of PD SCC cases showed strong expression and 50% sowed moderate expression of CD44. The chi square is 15.992. The *p*-value is 0.003. This result is significant at $p < 0.05$.

Table no 5: expression of CD44

	CD44 EXPRESSION	FREQUENCY	PERCENTAGE %
WD SCC (42)	STRONG	6	14.3%
	MODERATE	10	23.8%
	WEAK	26	61.9%
MD SCC (32)	STRONG	8	25%
	MODERATE	18	56.3%
	WEAK	6	18.7%
PD SCC (2)	STRONG	1	50%
	MODERATE	1	50%
	WEAK	0	0%

Bcl-2 was expressed as cytoplasmic positivity in all the cases of OSCC, with 44 cases (57.9%) showing weak positivity, 24 cases (31.6%) showing moderate grade of positivity and 8 cases (10.5%) showing strong Bcl-2 positivity.71.5% of WD SCC cases showed weak expression of BCL2, 46.9% of MD SCC cases showed moderate expression, 50% of PD SCC cases showed strong expression and 50% showed moderate expression of BCL2.The chi square is 11.3078. The *p*-value is 0.0233. This result is significant at $p < 0.05$

Table no 6 : expression of BCL2

	BCL2 EXPRESSION	FREQUENCY	PERCENTAGE %
WD SCC (42)	STRONG	4	9.5%
	MODERATE	8	19%
	WEAK	30	71.5%
MD SCC (32)	STRONG	3	9.4%
	MODERATE	15	46.9%
	WEAK	14	43.7%
PD SCC (2)	STRONG	1	50%
	MODERATE	1	50%
	WEAK	0	0%

Figure 1 : Well differentiated SCC H &E

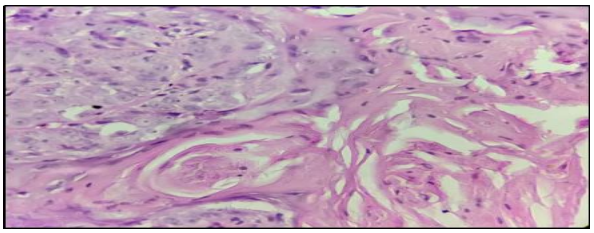


Figure 2 : Moderately differentiated SCC H & E

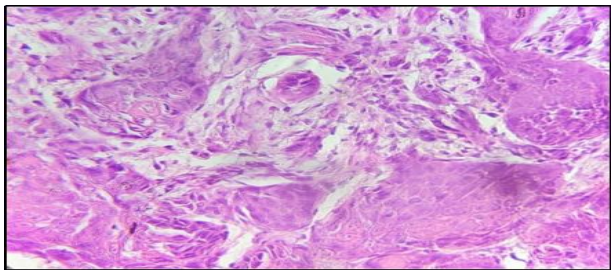
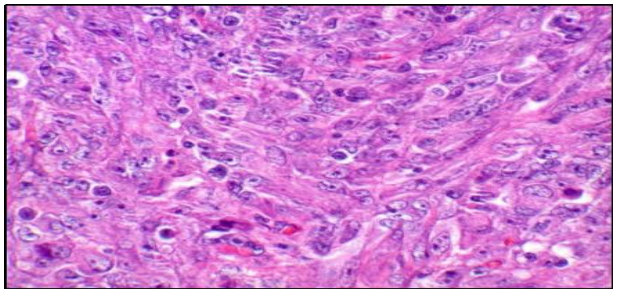


Figure 3 : Poorly differentiated SCC H & E



Figures 4-6 : Grades of CD44 expression in OSCC

Figure 4 : Weakpositivity

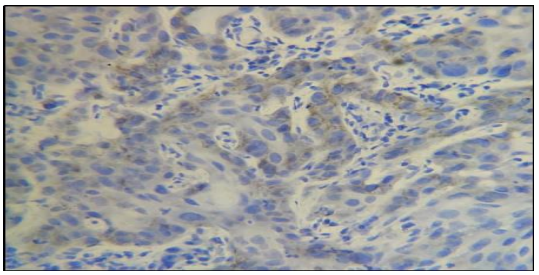


Figure 5 : Moderate positivity

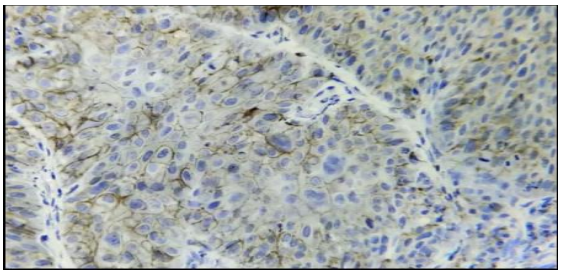
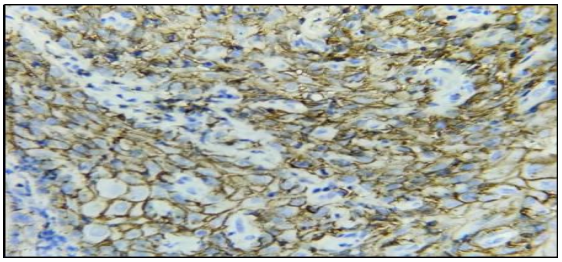


Figure 6 : Strong positivity



Figures 7-9 : Grades of Bcl-2 expression in OSCC

Figure 7 : Weakpositivity

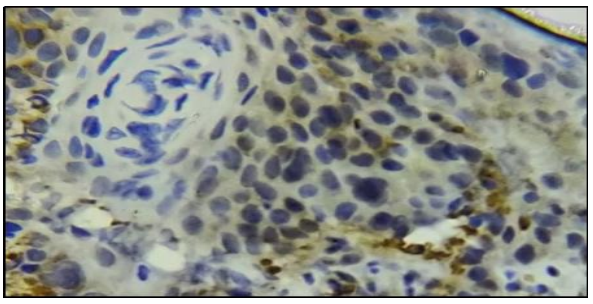


Figure 8 :Moderate positivity

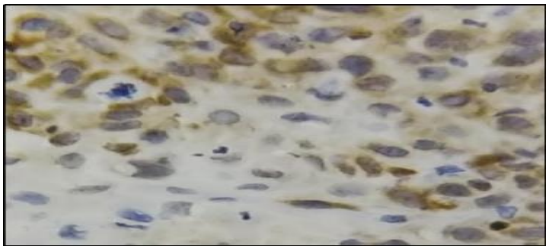
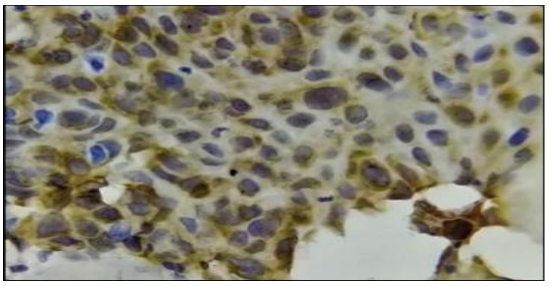


Figure 9 : Strong positivity



Discussion

Oral Squamous Cell Carcinoma (OSCC) is the most frequent malignant tumour of the oral cavity, exhibiting a heterogeneous behaviour. OSCC remains one of the most difficult malignancies to control because of its high propensity for local invasion and cervical lymph node dissemination[6]. The behaviour of OSCC is difficult to predict solely using conventional clinical and histopathological parameters, and due to location of the disease, the multi-modal tumour therapy usually prescribed leads to a reduction in quality of life, making the psychosocial consequences of OSCC greater than other malignancies. For these reasons, despite advances in therapeutic strategies, the survival rate of OSCC patients is still poor. Special attention has recently been focused on the use of potential molecular biomarkers as reliable predictors of tumour aggressiveness[7]. The assessment of prognostic biomarkers can also be useful in the selection of patients who would best benefit from intensive adjuvant therapy[8].

In the present study, incidence has been found to be most common in the age group of 41-60 years.Male to female ratio is 8.5 : 1. The CD44 family is a widely expressed transmembrane glycoprotein family that binds hyaluronic acid, growth factors, and ECM proteins. CD44 family consists of a standard form of CD44 (CD44s) and its alternative splice variants (CD44v). It is currently becoming evident that discrepancies in the OSCC ability for recurrence, loco regional or distant metastasis, as well as in the radio-resistance of its malignant cells, may be mainly due to the overexpression of a specific CD44 isoform. Bankfalvi et al. found that the increased immunoexpression of the CD44v9alternative splice isoform along with a loss of CD44s, v4, and v7 was significantlyassociated with a poorer clinical outcome in OSCC. In some other cases, theoverexpression of

CD44v (v3 and v6) appears to reflect the cellular invasivenessand leads to the increased aggressiveness of some HNSCCs, as well as that ofOSCCs, given that these different isoforms are also associated with lymph nodemetastasis and chemoresistance[9].

An increase in CD44 expression from well to moderate to poorly differentiated OSCC is seen in the present study, which correlates with the studies by Sanghravanian et al[10] and Jiajia Q et al[11]. Studies by Jiajia Q et al[11], Sanghravanian et al[10], Boxberg et al[12]and Kohei okuyama et al[13]demonstrated a statistically significant association between tumour grade of OSCC and the CD44 over-expression, correlating with the present study. However, studies by Kokko et al[14]showed no such statistically significant association between OSCC and its histological grade.

The expression and function of apoptosis regulating genes are under complex regulatory mechanisms including transcriptional control and translational modifications. The increased expression of Bcl-2 is not only essential for oral carcinogenesis but also influences the progression of the disease because it increases the survival rate of neoplastic cells, allowing new genetic mutations to occur and granting them higher resistance to chemotherapy and radiotherapy[8]. In the present study, 42.1% of cases which include mostly poorly differentiated and moderately differentiated OSCCs, show strong to intermediate staining, similar to the studies by Juneja s et al[15], Rahmani et al[16], Arya et al[17] where these variants showed strong to intermediate staining but constituted a minority of the total cases.

Stronger expression of Bcl-2 was seen in poorly differentiated OSCC, which is consistent with the findings of Juneja s et al[15], Rahmani et al[16], Chen Yu *et al*[4] and Sulkowska *et al*[18]. An increase in the expression of Bcl-2 from well to moderate to poorly differentiated OSCC was noted in the present study, which correlated with the studies by Sulkowska et al[18], Juneja s et al [15], Rahmani et al[16]. The present study correlated with the studies by Sulkoswska et al[18], Solomon et al[19], Arya et al[17],L yao et al[20] and Guan et al[21] which showed a statistically significant association between tumour grade of OSCC and Bcl-2 overexpression. However, studies by Rao roopa et al[22]showed no such statistically significant association between the OSCC and its histological grade.

Conclusion

Staging and grading continue to be among the most important parameters to understand the disease course and predict the prognosis of patients diagnosed with malignancies. This study was an endeavour to try and discover the association of CD44 and Bcl-2 expression with different grades of squamous cell carcinoma of oral cavity. The clinicopathological impacts of CD44s and its isoforms in promoting tumourigenesis suggest that CD44 may be a molecular target for cancer therapy. Therapeutic strategies that target CD44 or reduce CD44 expression are in various stages of clinical development. BCL-2 proteins, one of the most prominent anti-apoptotic proteins expressed in OSCC, contribute to cancer development and mediate resistance to current anticancer treatments. Several promising inhibitors of BCL-2 proteins have been developed in recent

years. Further studies on CD44 and Bcl-2 will be an important adjunct, along with lymph node metastasis to determine the prognosis and to design treatment options that would lead to lesser morbidity, and increase the survival rates of patients with squamous cell carcinoma of the oral cavity.

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