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Original Research Article

A STUDY ON CLINICO PATHOLOGICAL PROFILE AND CD44 EXPRESSION IN ORAL CAVITY AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA PATIENTS IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Oral cancer is more prevalent in developing countries and is primarily caused by tobacco use, unhealthy diets, physical inactivity, and infections. Understanding neoplastic transformation at the subcellular level is crucial for identifying and preventing oral cancer. This study aimed to evaluate the correlation between the clinical and pathological findings of CD44 expression in malignant cells and its prognostic impact in patients with oral squamous cell carcinoma. **Material and Methods**: This retrospective and comparative study was conducted using incisional and excisional biopsy specimens of the oral cavity at the Govt Stanely Medical College between August 2017 and August 2018. Of the 60 specimens, 20 were well-differentiated squamous cell carcinomas, 28 were moderately differentiated squamous cell carcinomas, and 12 were poorly differentiated squamous cell carcinomas, with or without nodal metastasis, were randomly selected for staining with the CD44 immunohistochemical marker. **Results**: Most patients were aged between 51 and 70 years (56.70%), with males comprising 76.70%. The mean depth of the tumours was 0.74 ± 0.38. T1 tumours were predominant (53.30%), with the majority having N0 nodes (65%). Grade 2 tumours accounted for the highest proportion (46.70%), and stage I tumours were the most prevalent (41.70%). Strong CD44 expression was observed in 73.30% of the cases, with 56.70% exhibiting basal cell invasion. Lesions were commonly found on the tongue (28.4%) and vocal cord (26.6%). There were no significant differences in age group, sex, tumour size, nodes, and basal cell invasion between the levels of expression ($p > 0.05$). There was a significant difference in the grade between expression levels ($p = 0.011$). **Conclusion**: Lower CD44 levels in aggressive OSCC (poor differentiation, lymph node spread) suggest a link to progression. CD44 might maintain tissue structure, and its loss could be a poor prognostic marker.

INTRODUCTION

Oral cancer is more common in developing countries than in developed countries. It may arise as a primary cancer in any of the oral tissues. It includes neoplasms arising from the oral cavity, including the buccal mucosa, tongue, lips, hard and soft palate, oropharynx, larynx, and hypopharynx, excluding neoplasms of the salivary gland. In India, the age-standardised incidence rate of oral cancer is 12.6 per 100,000 people.[1] The prevalence of oral cancers among men is high, ranking among the three most common types of cancer. The incidence is increasing owing to the combined effect of the aging of the population and increasing prevalence of cancer risk factors. It has been estimated that the increased prevalence is due to tobacco, unhealthy diet, physical inactivity and infections.[2]

Tobacco use and excessive alcohol consumption are coexisting factors that have been estimated to account for approximately 90% of oral cavity cancers. The increased use of smokeless tobacco causes oral cancer was recently been confirmed by the International Agency for Research on Cancer.[3] Oral squamous cell carcinoma constitutes more than

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90% of oral malignancies. The 5-year survival rate for oral carcinomas is about 55%, despite the therapeutic advances and also considerably reduced for more posteriorly located tumours.[4] The recent WHO has taken resolutions on a diet, physical activity and health for the control and prevention of oral malignancy.[5]

Oral squamous cell carcinogenesis involves many processes, including genetic events, that affect the normal function of oncogenes and tumour suppressor genes. Because of the increased incidence and high recurrence rate of oral squamous cell carcinomas which in turn has increased morbidity and mortality, it is very important to understand the neoplastic transformation at a sub-cellular level.[6] The earliest morphologic alterations that could appear as premalignant lesions include leukoplakia and erythroplakia. 50% of leukoplakias exhibit dysplasia and have a potential for malignant transformation for about 0.1-2% per year.[7]

A combined study of relevant clinical data, adequate sampling, detailed histopathological examination, and techniques such as immunohistochemistry is important to identify neoplastic and non-neoplastic lesions and neoplastic lesions with high malignant potential for invasion and metastasis. Many studies have been conducted to establish various prognostic factors for oral SCC at the molecular level, including various tumour suppressor genes that have been identified in the aetiology of oral and oropharyngeal squamous cell carcinoma.[8,9] The development of malignancy appears to require aberrations in cell death machinery and cell-cell and/or cell-matrix interactions that cooperate with cell cycle defects.[10] CD44 is a major human cell surface receptor for hyaluronate and functions in a diverse range of physiological processes. CD44 plays a role in stimulating the in vivo aggressiveness of tumours through hyaluronate-rich stroma.[11]

Aim

This study aimed to evaluate the correlation between the clinical and pathological findings of CD44 expression in malignant cells and its prognostic impact in patients with oral squamous cell carcinoma.

MATERIALS AND METHODS

This retrospective and comparative study was conducted using incisional and excision biopsy specimens of the oral cavity obtained from the Department of Oto Rhinolaryngology and Surgery and reported by the Department of Pathology, Govt Stanely Medical College, Chennai, between August 2017 and August 2018. The study was approved by the institutional ethics committee before initiation, and informed consent was obtained from all patients.

Inclusion Criteria

All oral and oropharyngeal squamous cell carcinomas diagnosed by incision/excision biopsy were sent from the Department of Surgery and Otorhinolaryngology, Govt Stanley Medical College, and all Histopathological variants of Oral Squamous Cell Carcinoma reported in the Department of Pathology, Govt Stanley Medical College between August 2017 and August 2018 were included.

Exclusion Criteria

Benign neoplasms arising from the oral and oropharyngeal mucosa, primary malignancies of the oral cavity and oropharynx other than squamous cell carcinoma, and recurrent and metastatic tumours of the oral cavity and oropharynx were excluded from the study.

All cases were selected using simple randomisation. Of all the specimens, 60 were randomly selected, of which 20 were well-differentiated squamous cell carcinomas, 28 were moderately differentiated squamous cell carcinomas, and 12 were poorly differentiated squamous cell carcinomas, with or without nodal metastasis, were randomly selected for staining with the CD44 immunohistochemical marker. Group 1: specimen diagnosed as malignant by Histopathological Examination, Grade I, Group 2: specimen diagnosed as malignant by Histopathological Examination, Grade II, and Group 3: specimen diagnosed as malignant by Histopathological Examination, Grade III.

A detailed history regarding the patient's age, sex, site, and personal history was collected from the surgical pathology records. Hematoxylin and eosin-stained tissue sections were used to confirm the histological diagnosis of squamous cell carcinoma. The specimens were subjected to excision/incision biopsy.

Histopathological Evaluation

Sections of 4-micrometer thickness were obtained from the corresponding paraffin blocks using a semi-automated microtome with disposable blades, followed by staining with Haematoxylin and Eosin. The stained sections were reviewed. The histological characteristics of oral squamous cell carcinoma were classified into well, moderately, and poorly differentiated groups (G1–G3) with or without nodal metastasis according to the criteria proposed by the World Health Organization.

As of January 2018, the American Joint Committee on Cancer (AJCC) staging system has been used for oral cavity and oropharyngeal cancers. It involves TNM classification based on tumour extent (T), metastasis to nearby lymph nodes (N), and distant metastasis (M). Corresponding paraffin blocks were retrieved, and sections were cut at 4-micrometer thickness using a semi-automated microtome with disposable blades. Sections were mounted on chrome alum-coated slides, subjected to antigen retrieval using a pressure cooker technique with TRIS buffer solution at pH 9.2 and treated with horseradish peroxidase (HRP) for analysis.

Statistical Analysis

Tumour size, depth, stage, and nodes were considered as outcome variables, and the level of

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expression was considered as an explanatory variable. All Quantitative variables (age and depth) were checked for normal distribution within each category of explanatory variables by visual inspection of histograms and normality Q-Q plots. The Shapiro-Wilk test was also conducted to assess normal distribution. The Shapiro-Wilk test ($p > 0.05$) was considered to be normally distributed.

For normally distributed quantitative parameters (age), the mean values were compared between the expression levels using an independent sample t-test. For non-normally distributed quantitative parameters, (depth) median and interquartile range (IQR) were compared between expression levels using the Mann-Whitney U test. Categorical outcomes, gender, stage, nodes, and timer size were compared between the levels of expression using the chi-square test. Statistical significance was set at $p < 0.05$. IBM SPSS version 22 was used for the statistical analysis.

RESULTS

The mean age was 55.08 ± 12.14, the minimum age was 31 years, and the maximum age was 80 years in the study population (95% CI 51.95 58.22). Among the study population, 20 (33.30%) were aged between 30 and 50 years, 34 (56.70%) were aged between 51 and 70 years, and the remaining six (10%) were aged 71 years and above. 46 (76.70%) patients were males and the remaining 14(23.30%) were females. The mean Depth was 0.74 ± 0.38, the minimum level was 0.30 and the maximum level was 2.00 in the study population (95% CI 0.65 to 0.84).

32(53.30%) patients had T1 tumours, 24(40%) had T2 tumours, and 4(6.70%) had T3 tumours. The majority of the study population consisted of 39(65%) patients with N0 nodes, 12(20%) with N1 nodes, and 4(6.70%) with N2b nodes. Twenty (33.30%) people were in grade 1, 28(46.70%) were in grade 2, and the remaining 12(20%) were in grade 3. 25(41.70%) patients were in stage I, 12(20%) were in stage II, 18(30%) were in stage III, and the remaining 5(8.30%) were in stage IV.

Among the study population, 16(26.70%) had weak expression levels and the remaining 44(73.30%) had strong expression levels. 34(56.70%) patients had basal cell invasion, and the remaining 26(43.30%) patients had no basal cell invasion. Seventeen (28.4%) patients had lesions on the tongue, 16(26.6%) had lesions on the vocal cord, 11(18.4%) had lesions on the buccal mucosa, 9(15%) had lesions on the pharynx, and 7(11.6%) had lesions on the lip. [Table 1]

The mean age in the weak-level expression group was 53.63 ± 10.6, it was 55.61 ± 12.72 for the strong-level expression group. There were no significant differences in age group, sex, tumour size, nodes, and basal cell invasion between the levels of expression ($p > 0.05$).

In the weak expression group, 2 (12.5%) were grade 1, 7 (43.8%) were grade 2, and 7 (43.8%) were grade 3. In the strong expression group, 18 (40.9%) were grade 1, 21 (47.7%) were grade 2, and 5 (11.4%) were grade 3. There was a significant difference in the grade between the expression levels ($p = 0.011$). [Table 2]

Table 1: Demographic data of the study

		Frequency	Percentage
Age group (Years)	30 to 50	20	33.30%
	51 to 70	34	56.70%
	71 and above	6	10.00%
Gender	Male	46	76.70%
	Female	14	23.30%
Tumour size	T1	32	53.30%
	T2	24	40.00%
	T3	4	6.70%
Nodes	N0	39	65.00%
	N1	12	20.00%
	N1A	3	5.00%
	N1B	1	1.70%
	N2	1	1.70%
	N2b	4	6.70%
Grade	Grade 1	20	33.30%
	Grade 2	28	46.70%
	Grade 3	12	20.00%
Stage	I	25	41.70%
	II	12	20.00%
	III	18	30.00%
	IV A	5	8.30%
Level of expression	Weak	16	26.70%
	Strong	44	73.30%
Basal cell invasion	Present	34	56.70%
	Absent	26	43.30%
Site of lesion	Tongue	17	28.40%
	Vocal cord	16	26.60%
	Buccal mucosa	11	18.40%

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	Pharynx	9	15.00%
	Lip	7	11.60%

Table 2: Comparison of level of expression with clinical and pathological findings

		Level of expression		P-value
		Weak (N=16)	Strong (N=44)	
Age group	30 to 50	6 (37.5%)	14 (31.8%)	0.81
	51 to 70	9 (56.3%)	25 (56.8%)	
	71 and above	1 (6.3%)	5 (11.4%)	
Gender	Male	14 (87.5%)	32 (72.7%)	0.232
	Female	2 (12.5%)	12 (27.3%)	
Tumour size	T1	12 (75%)	20 (45.5%)	0.111
	T2	3 (18.8%)	21 (47.7%)	
	T3	1 (6.3%)	3 (6.8%)	
Nodes	N0	9 (56.3%)	30 (68.2%)	0.643
	N1	5 (31.3%)	11 (25%)	
	N2	2 (12.5%)	3 (6.8%)	
Grade	Grade 1	2 (12.5%)	18 (40.9%)	0.011
	Grade 2	7 (43.8%)	21 (47.7%)	
	Grade 3	7 (43.8%)	5 (11.4%)	
Stage	I	9 (56.3%)	16 (36.4%)	-
	II	0 (0%)	12 (27.3%)	
	III	5 (31.3%)	13 (29.5%)	
	IV A	2 (12.5%)	3 (6.8%)	
Basal cell invasion	Present	6 (37.5%)	28 (63.6%)	0.071
	Absent	10 (62.5%)	16 (36.4%)	

DISCUSSION

In our study, clinicopathological and immunohistochemical evaluations were performed for 60 cases of oral and oropharyngeal SCC. An attempt was made to assess the significance of CD 44 expression and its correlation with prognosis, basal invasion, and grading so that targeted therapy can be attempted to improve prognosis. Our study showed that the incidence of OSCC and OPSCC ranged from the 3rd to the 8th decade. The highest incidence was noted in 5th decade of life. The mean age at presentation was 55.08, with a male predominance of 76.70% compared with females, with a distribution of approximately 23.30%. Similarly, Selvamani et al. showed that the mean age of presentation is 55.75 with a range from 27-80 years, and increased incidence among males than females.[12] Singh et al. showed the mean age of presentation was 52.77.[13]

In our study, the most common site was the tongue (28.4%), followed by the vocal cords, buccal mucosa, pharynx, and lips. In a comparative study, Selvamani et al. showed that incidence is common among tongues at 32%.[12] Rai et al. showed that the common site was buccal mucosa with 53.1%, tongue with 17.7%, and 13.8% for lip tumours.[14] In a study by T. Smitha et al., the percentages for a tongue, buccal mucosa, and lip tumours were 12.29%, 30.90%, and 3.99% respectively.[15]

In our study, out of 60 cases, 20 (33.3%) were reported as well-differentiated squamous cell carcinoma, 28 (46.7%) cases were reported as moderately differentiated squamous cell carcinoma, and the remaining 12 (20%) cases were reported as poorly differentiated squamous cell carcinoma. In a study by Rai et al., the percentages were 50.8% for well-differentiated SCC, 29.20% for moderately differentiated SCC, and 20% for poorly differentiated tumours.[14] In our study, moderately differentiated SCC was increased in number compared to well-differentiated SCC. This was not in agreement with other studies, in which well-differentiated carcinoma was the most common type.

In our study, of the 60 cases, 21(35%) presented with nodal metastasis, and the remaining 39(65%) presented without nodal metastasis. This was in concordance with Xia et al., where most cases were nodal negative, which is 10 cases with nodal metastasis present, while 34 cases had absent nodal metastasis. This indicates that patients are aware of OSCC and OPSCC and present to the clinician in the early stage.[16]

In our study, 32 out of 60 cases presented at the T1 stage,24 cases were at the T2 stage, 4 cases were at the T3 stage, and none of the cases were presented at the T4 stage. Thus, the majority of cases were in the T1 stage. This was not in agreement with other studies where T2 was the most common stage of presentation. The association between tumour stage and CD44 immunoexpression was not statistically significant. However, in stage T2, almost all cases showed strong CD44 immunoexpression. The association between nodal status and CD44 immunoexpression was not significant. Strong expression of CD44 was observed in both the N0 and N1 categories. This was in agreement with another study by Hema et al., who also reported no significant difference in tumour stage and nodal status based on CD44 immunoexpression.

In our study, among the 20 cases of well-differentiated SCC, overall CD44 expression was present in 20 cases in strong and weak forms, in which 18 cases had strong expression and 2 had weak expression. In 28 cases of moderately

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differentiated SCC, 21 showed strong expression and 7 showed weak expression; in 12 cases of poorly differentiated SCC, weak expression was observed in 7, and strong expression was observed in 5 cases. Thus, well-differentiated carcinomas show increased expression of CD44 compared to poorly differentiated carcinomas. This was similar to Hema et al. and Kaza et al. shows a statistically significant outcome with a p-value of 0.011.[17,18]

CONCLUSION

This study revealed a statistically significant inverse correlation between CD44 expression, histological grade, tumor stage, and lymph node status in OSCC. Tumors with strong CD44 staining (well-differentiated) exhibited better cell adhesion, potentially indicating a role in maintaining tissue architecture. Conversely, lower CD44 expression suggests a link to tumor progression and metastasis.

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