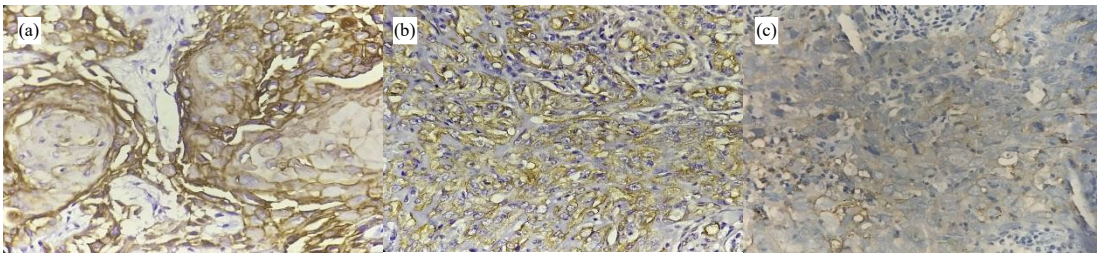

Figure 2 (a) WDSCC: Shows nests of tumour cells with membranous positivity of E-cadherin (3+) (IHC E-cadherin X 400); (b) MDSCC: Shows both membranous and cytoplasmic positivity of E-cadherin (2+). (IHC E-cadherin X 400); (c) PDSCC: Shows cytoplasmic staining (1+) (IHC E-cadherin X 400)

β-catenin immunoreactivity score decreased as the tumour grade increased from well to poorly differentiated carcinoma. β-catenin immunoreactivity was high in 77.14% cases of well differentiated carcinoma and low in 22.86% cases. None of the case showed negative immunostaining. While in poorly differentiated carcinoma, 81.82% cases showed low immunoreactivity and 9.09% cases each showed high and negative immunostaining. Thereby stating the fact that as the tumour progresses to a lower grade, there is loss of β-catenin expression (Table 6).

Table 6 β-catenin expression in different grades of OSCC

Grade of OSCC	β-catenin Immunoreactivity Score								p-value
	Negative (0)		Low (1-4)		High (5-12)		Total		
	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	
Well	0	0	8	22.86	27	77.14	35	100	p<0.001
Moderately	3	12	14	56	8	32	25	100	
Poorly	1	9.09	9	81.82	1	9.09	11	100	
Total	4	5.63	31	43.66	36	50.7	71	100	
p-values: a:b <0.01; a:c <0.001; b:c >0.05									

A statistical significant difference in β-catenin expression was noted on comparing well differentiated carcinoma with moderately differentiated carcinoma, p-value<0.01; and well differentiated carcinoma with poorly differentiated carcinoma, p-value<0.001. However, no statistical significance (p-value>0.05) was found in β-catenin expression amongst moderately differentiated carcinoma and poorly differentiated carcinoma.

Table 7 β-catenin staining pattern/location in different grades of OSCC

Staining Location	Grade of OSCC								p-value
	WDSCC		MDSCC		PDSCC		Total		
	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	
Membranous	28	80	13	52	0	0	41	57.75	p<0.001
Both Membranous and Cytoplasmic	7	20	10	40	2	18.18	19	26.76	
Cytoplasmic	0	0	1	4	2	18.18	3	4.23	
Absent	0	0	1	4	7	63.64	8	11.27	
Total	35	100	25	100	11	100	71	100	

Table 7 depicts, 27 (77.14%) cases of well differentiated carcinoma (Figure 3a) showed membranous staining of β-catenin as compared to 8 (32%) cases of moderately differentiated and 1 (9.09%) case of poorly differentiated carcinoma. Both membranous and cytoplasmic staining was seen in 8 (22.86%) cases of well differentiated carcinoma, 11 (44%) cases of moderately differentiated carcinoma (Figure 3b) and 3 (27.27%) cases of poorly

differentiated carcinoma. 3 (12%) case of moderately differentiated and 5 (45.45%) cases of poorly differentiated carcinoma had cytoplasmic staining. 1 (9.09%) case of poorly differentiated carcinoma showed nuclear staining. While 3 (12%) cases of moderately differentiated carcinoma and 1 (9.09%) case of poorly differentiated carcinoma showed absent staining (Figure 3c). Thereby indicating that as the tumour grade increases from well to poorly differentiated OSCC, β-catenin membranous and cytoplasmic positivity decreases and nuclear positivity increases. Chi square test showed a p-value of <0.001, which was statistically significant.

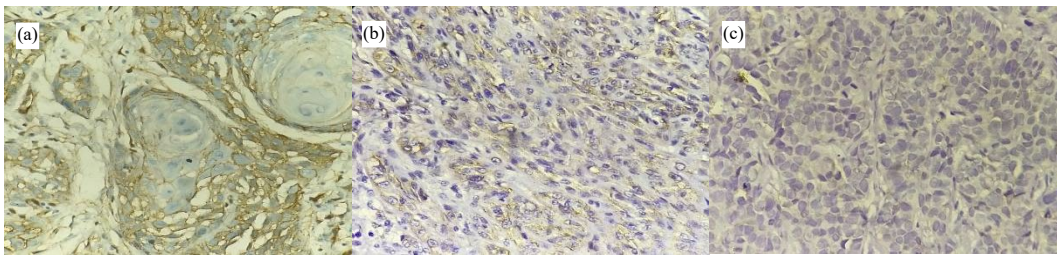

Figure 3(a) WDSCC: Shows membranous positivity of β-catenin (2+) in tumour cells (IHC β-catenin X 400); (b) MDSCC: Shows both membranous and cytoplasmic positivity of β-catenin (1+). (IHC β-catenin X 400); (c) PDSCC: Shows absence of staining (IHC β-catenin X 400)

In our study, lymphnode metastasis was present in 29 (40.8%) cases of OSCC. Out of these, 19 (26.8%) cases showed low immunoreactivity and 10 (14.1%) cases showed high immunoreactivity for E-cadherin expression which was statistically significant. While, 15 (21.1%) cases showed low immunoreactivity and 14 (19.7%) cases with high immunoreactivity for β-catenin expression, which was statistically non-significant. The present study showed a statistically significant difference in the expression of E-cadherin in patients who had lymph node metastasis showing decreased expression in these patients, indicating loss of E-cadherin is associated with more lymph nodes metastasis and more invasions. However, there was no statistically significant expression of β-catenin in patients with lymph node metastasis.

Table 8 Comparision of E-cadherin and β-cateninimmunoexpression in OSCC with or without lymphnode metastasis

Groups	E-cadherin		p-value	β-catenin		p-value
	≤ 4 (%)	5 to 12 (%)		≤ 4 (%)	5 to 12 (%)	
OSCC without Lymphnode metastasis [n=42]	16 (22.5)	26 (36.6)	0.031	20 (28.2)	22 (31.0)	0.733
OSCC with Lymphnode metastasis [n=29]	19 (26.8)	10 (14.1)		15 (21.1)	14 (19.7)	

This table showed that there was a significant difference in E- cadherin expression at the primary site of OSCC, with or without lymph node metastases but no significant difference observed in β-catenin expression (Table 8).

DISCUSSION

Adhesion molecules play a central role in pathogenesis and progression of malignant tumours [11]. Therefore, it is important to evaluate the role of cell adhesion molecules like E-cadherin and β-catenin in tumour metastasis of oral squamous cell carcinoma [12].

In the present study, we evaluated the immune-histochemical expression of E-cadherin and β-catenin in oral squamous cell carcinoma, and compared its change with different grades of tumour, age, gender, site, lymph node metastasis etc.

In our study, we found WDSCC constituted the maximum number of cases (48.3%) followed by MDSCC (45.6%); Ahluwalia *et al.*, 2001 found 65.97% well differentiated, 24.49% moderately differentiated, and 9.53% poorly differentiated squamous cell carcinoma [13]. Similarly, Patel *et al.*, 2004 also found 60.12% well differentiated, 38.7% moderately differentiated, and 1.18% poorly differentiated squamous cell carcinoma [14].

Our study revealed increase prevalence of OSCC in the males with 161 cases (89.4%) as compared to females with only 19 cases (10.6%) with male to female ratio of 8.5:1. Sharma *et al.*, 2010 have also documented a male to

female ratio of 2.2:1 in OSCC cases in their study [15]. Cervical lymph node metastasis was observed in 52 cases (28.9%) of OSCC. Singh *et al*., 2003 have demonstrated that metastatic behaviour was critical to survival, since patients with distant disease have three times less five-year survival rate than patients with local spread to lymph nodes [16].

In our study, degree of E-cadherin and β-catenin expression decreased as the grade of the tumour was increased and depicts a poor prognosis. Similar findings were seen in a study conducted by Mehendiratta *et al*., 2014 on Indian population where they observed absent E-cadherin membranous staining in 0%, 10% and 30% cases of well, moderately and poorly differentiated oral squamous cell carcinomas respectively. It was concluded that loss of E-cadherin expression at the invasive tumour front is an important event in the progression of oral squamous cell carcinomas and tumours with a loss of expression of E-cadherin are those which had a poor prognosis [17]. Afrem *et al*., 2014 documented a decrease E-cadherin reactivity in parallel with decreasing tumour differentiation and with the increase of invasion pattern of tongue carcinoma [18]. Similar study done by Zaid *et al*., 2014 also documented β-catenin expression in different histological grades of oral squamous cell carcinoma and reported the expression of β-catenin in 87.3% of the cells in WDOSCC, 68.7% of MDSCC and 43.4% of PDSCC [19].

In the present study, we analysed that E-cadherin expression changed from membranous to cytoplasmic to complete absrent staining pattern as tumour grade increased from well to poorly differentiated oral squamous cell carcinoma. A study by Kaplanis *et al*., 2004 showed loss of membranous staining and a progressive increase in cytoplasmic staining of E-cadherin, as the disease progressed from dysplasia to carcinoma in situ to invasive squamous cell carcinoma [20]. Another similar study by Mattijssen *et al*., 1993 however, found no correlation between tumour differentiation and E-cadherin expression [21].

Similarly, β-catenin membranous and cytoplasmic positivity decreased and nuclear positivity increased as the tumour grade increased from well to poorly differentiated OSCC. Our findings were in concordance with the study done by Barakat *et al.*, 2015 who analysed more than 56% of the tumour cells in the whole sample showed abnormal expression for β-catenin, in 14.7% cytoplasmic expression was noticed whereas the nuclear expression appeared in 5% of the cells [22]. Zhi-gang *et al*., 2008 also found different degrees of reduced expression of β-catenin at the cell membrane in 54 (71%) cases with squamous cell carcinoma. Cytoplasmic β-catenin expression was seen in 17 tumors (22.4%). Three cases were found with nuclear β-catenin expression. The cytoplasm and nuclear β-catenin expression was only seen in the tumours with poor differentiation. They concluded that reduced expression of β-catenin may constitute a hallmark of aggressive biological behaviour of squamous cell carcinoma [23].

Study done by Balasundaram *et al*., 2014 who also analysed the down regulation of molecular markers E-cadherin and β-catenin in oral squamous cell carcinoma. However, they did not show any significant difference in the expression of E-cadherin as well as β-catenin in oral squamous cell carcinoma with and without lymphnode metastases [24]. It is likely that these discrepancies may be due to variation in the experimental model or tissue used, clinico-pathological characteristics of the patients, sample size, and methodology.

There was a direct correlation of E-cadherin and β-catenin expression in oral squamous cell carcinoma. Anneroth *et al*., 1986 and Crissman *et al*., 1984, have shown a positive correlation between the degree of histological malignancy and prognosis of the disease [25,26]. So we may suggest that the reduced expression of E-cadherin and β-catenin is an indicator of a poor prognosis in oral squamous cell carcinoma. Loss of expression of both E-cadherin and β-catenin are frequent events in oral squamous cell carcinoma of all histological grades, and both proteins are thus likely participants in the pathogenesis of oral squamous cell carcinoma.

CONCLUSION

The main characteristics of oral squamous cell carcinoma are invasion and the tendency to metastasize. Escape from the primary tumour site is the initial step of invasion and metastasis of cancers by the disruption of normal cell-cell adhesion. The major goal of cancer research is to identify the crucial pathways involved in the growth regulation of normal cells and to understand how tumour cells escape these control mechanisms. Thus, the present study concluded that loss of expression of both the proteins is of diagnostic and prognostic significance with respect to the histologic grades of OSCC.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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