Expression of E-Cadherin in Association with Histological Prognostic Parameters of Oral Squamous Cell Carcinoma; A Retrospective Analysis

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Abstract

Background: Several histological prognostic parameters of oral squamous cell carcinoma have been studied over the past decades and Anneroth grading system was found to have better prognostic value in this regard. During cancer progression, loss of E-cadherin expression is a crucial step and is associated with invasion and metastasis. **Objective**: This study aimed to evaluate the histological prognostic parameters and also to find the association of E-cadherin expression with these parameters.

Methods: A total of 61 histologically diagnosed cases of oral squamous cell carcinoma (OSCC) were selected for this study. Among these, 36 cases were resected tumor specimens and 25 were oral mucosal biopsies. Representative sections from each paraffin block were selected for routine histomorphological study and for immunohistochemistry with E-cadherin antibody.

Result: In this study, an inverse correlation of E-cadherin expression was found with nuclear pleomorphism, pattern of invasion, stage of invasion and total Anneroth grade which means that there is a significant linear relationship of loss of E-cadherin with worsening of these parameters. The correlation E-cadherin expression with pattern of invasion was the strongest among all the parameters evaluated here. On the other hand, no association of E-cadherin was found with WHO histological grading, degree of keratinization, number of mitoses, lymphoplasmacytic response and lymph node metastasis.

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Keywords: E-Cadherin, Anneroth grading, Oral squamous cell carcinoma

Introduction

Oral squamous cell carcinoma (OSCC) ranks as one of the top ten cancers worldwide. The prognosis of advanced oral squamous cell carcinoma (stage III and IV) is poor. Current treatment protocol for oral squamous cell carcinoma is based on surgery or radiation, with or without concomitant chemotherapy. The 5-year survival rate of oral squamous cell carcinoma couldn't have been increased over the past four decades because of frequent relapse and cervical lymph node metastasis.[1] Survivors also suffer from multiple treatment complications like oral mucositis, hyposalivation, osteoradionecrosis, tissue fibrosis, morbidity from jaw resection, disfigurement and loss of function that further diminish the quality of life.[2]

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Current histologic grading system of OSCC is based on WHO (1971) defined morphologic criteria of the tumour. A. C. Broder primarily developed this quantitative grading of cancer in 1925.[3] This grading system has been shown to be of limited value in regard to prognosis and therapy. The reason behind this lacking may be that this system evaluates only the tumor cell population. It doesnot consider the biologic activity of the tumor in relationship to the surrounding host tissue. In order to include both the tumor cell population and the tumor-host relationship, Anneroth[4] et al developed a multifactorial histologic grading system including six morphological parameters. These are degree of keratinization, nuclear pleomorphism, number of mitoses, pattern of invasion, stage of invasion and lymphoplasmacytic infiltration. According to Crissman[5] et al pattern of invasion was the single most important histologic variable in predicting patient survival. Four types of pattern of invasion was described in Anneroth grading system (Table I).[6]

Oral carcinogenesis is a multistep genetic process. Around six to ten genetic events are believed to accumulate to result in oral carcinogenesis.[7] Cell surface adhesion molecules have an important role in inhibition of oral keratinocyte proliferation and angiogenesis. Cadherin is a superfamily of calcium-mediated membrane glycoproteins and cell surface adhesion molecules. Some common cadherins expressed by epithelial cells are E-cadherin, N-cadherin, and P-cadherin. Among these, epithelial (E-cadherin) cadherin plays a pivotal role in maintaining epithelial integrity. It binds to cytosolic proteins namely α-catenin, β-catenin, and, γ-catenin which in turn links to the actins to form the intracytoskeleton.[8] However, it has been shown that E-cadherin also regulates important cell signaling pathways and influences mechanisms of proliferation, differentiation, as well as apoptosis.[9] Moreover, it mediates the cadherin/catenin complex interaction with epidermal growth factor receptor (EGFR).[10] Dysfunctional E-cadherin mediated cell adhesion has been suggested to be associated with the loss of differentiation and acquisition of an invasive phenotype. In most cancers of epithelial origin, including oral squamous cell carcinoma, E-cadherin mediated cell-cell adhesion is lost concomitantly with progression towards malignancy.[11] Decreased E-cadherin expression in cancerous tissue also correlates with the poor prognosis or outcome of OSCC patients.[12] Appropriate drugs for restoration of E-cadherin expression in early stages can be a novel therapeutic option to prevent invasiveness as well as metastasis. Restoration of E-cadherin expression can be achieved by using demethylating drugs for example 5′-azacytidine.[13] Recently there are some ongoing clinical trials on the usefulness of targeted therapy like EGFR tyrosine kinase inhibitors in oral squamous cell carcinoma patients. Some recent studies suggest that restoration of E-cadherin also increases the sensitivity of EGFR tyrosine kinase inhibitors in oral squamous cell carcinoma as well as other surface epithelial cancers.[14, 15] For this reason, this study is designed to see the expression of E-cadherin in OSCC cases and to correlate this expression with the histological prognostic parameters.

Methods

This was a retrospective observational study. A total of 61 histologically diagnosed cases of OSCC were selected for the study. Among these, 36 cases were resected tumor specimens and 25 were oral mucosal biopsies. These cases had been diagnosed previously as squamous cell carcinoma from January 2015 to September 2017 at the Pathology Department of BSMMU and a private histopathology diagnostic laboratory in Dhaka city. Corresponding paraffin blocks were

collected from the archives of both the above mentioned laboratories.

Histomorphological study

Hematoxylin & eosin stained sections of each case were reviewed to confirm the histological diagnosis. Then, all the cases were categorized into well, moderate and poorly differentiated groups according to WHO defined criteria (1971).

Each of the six individual morphological parameters of Anneroth's grading system[4] (Table I) were evaluated in 36 cases which included completely excised tumor. As because the 5th parameter, stage of invasion, was not applicable to mucosal biopsy, this could not be evaluated in 25 oral mucosal biopsy specimens. In these cases, rest of the five morphological parameters were evaluated.

Table I: Anneroth et al (1987) multifactorial grading/scoring system for OSCC

	Morphologic parameter	Points/Scores			
		1	2	3	4
1	Degree of keratinization	>50% cells keratinized	20-50% cells keratinized	5-20% cells keratinized	0-5% cells keratinized
2	Nuclear pleomorphism	Little nuclear pleomorphism	Moderately abundant nuclear pleomorphism	Abundant nuclear pleomorphism	Extreme nuclear pleomorphism
3	Number of mitoses/HPF	0-1	2-3	4-5	>5
4	Pattern of invasion	Pushing, well-delineated infiltrating borders	Infiltrating, solid cords, bands and/or strands	Small groups or cords of infiltrating cells (n>15)	Marked and wide-spread cellular dissemination in small groups and/or in single cells (n<15)
5	Stage of invasion	Carcinoma-in-situ and/or questionable invasion	Distinct invasion, but involving lamina propria only	Invasion below lamina propria adjacent to muscles, salivary gland tissues, and periosteum	Extensive and deep invasion replacing most of the stromal tissue and infiltrating jaw bone
6	Lymphoplasmacytic infiltration	Marked	Moderate	Slight	None

Total Annerothgrade: Score 6-12: Grade I, Score 13-18: Grade II, Score 19-24: Grade III

Each case got a separate scoring point for every individual parameter (Table 1). Total Anneroth grading was done in the 36 resected specimens which fulfilled the criteria for evaluation of all of the six morphological parameters.After summation of the separate scoring points, these cases were also classified into grade I (6-12), II (13-18) and III (19-24). Among the 36 resected specimens, only 21 cases included lymph nodes with the excised tumor. So lymph node status could be evaluated in these 21 cases only. Cases with lymph nodes were divided into metastatic and non-metastatic groups depending on the presence of lymph node metastasis.

Immunohistochemical Study

From paraffin-embedded blocks, 5-micrometer thick sections were cut, deparaffinized with xylene and rehydrated through a graded series of alcohol. For antigen retrieval, the samples were treated with Dako Target Retrieval solution. Solutions were taken in coplin jar and pre-heated in the water bath at 65^0C. Then slides were kept in this solution and heated in the water bath at 95-99^0C for 30-40 minutes. Then the sections were stained with monoclonal mouse anti-human E-cadherin antibody (Clone NCH-38). Immunostaining was done using Dako Autostainer Plus at the immunohistochemistry laboratory, department of Pathology, BSMMU. For E-cadherin immunostain, positive control was taken from sections of normal breast tissue.

The expressions of E-cadherin were semi-quantitatively analyzed in at least ten microscopic fields at the invasive fronts of the tumor. The mean expression of these ten microscopic fields has been evaluated. Two parameters have been considered in case of this evaluation proposed by Scholtenet al.[16] These are: Staining intensity and percentage of positivity in tumor cells.

Regarding the staining intensity, cases were analyzed under light microscopy (×400 magnification), using the following 4-point scoring system:0 (absent), 1 (weak), 2 (moderate) and 3 (strong). According to percentage of immunopositive cells, cases were scored as: 0 = 0, 1=1–25%, 2 = 26–50%, 3 = 51–75% and 4 =>75%. The degree of membranous staining was calculated as the sum of staining intensity and positivity in tumor cells at the invasive front of the tumour (range 0–7). Total staining score of 5 or more was considered positive and 4 or less as negative.The statistical analysis was carried out using the Statistical Package for Social Sciences for Windows (SPSS Inc., Chicago, Illinois, USA), Version 24. The correlation between WHO (1971) histological grading and E-cadherin expression, each of the six morphological parameters and E-cadherin expression, total Anneroth grade and E-cadherin expression were evaluated using Spearman rank correlation co-efficient test. The value of the correlation co-efficient r_s denotes the strength of correlation.The association between lymph node metastasis and E-cadherin expression was evaluated using Chi-Square (X^2) test. P value <0.05 was considered as statistically significant.

Results

Total 61 cases were included in this study comprising of 17 hemimandibulectomy specimens, 3 maxillectomy specimens, 16 excised tumors and 25 oral mucosal biopsies. Ages of the patients ranged from 28 to 100 years. The subjects were grouped on the basis of decades and the highest number of cases (32.8%) belonged to 51-60 years age group. Among 61 cases, 32 were female and 29 were male with a male/female ratio of 1:1.1.

The correlation between expression of E-cadherin and histological prognostic parameters are summarized in Table II. This

study found an inverse correlation of E-cadherin expression with nuclear pleomorphism, pattern of invasion, stage of invasion and total Anneroth grade, which means that there is a significant linear relationship of loss of E-cadherin with worsening of these parameters. Pattern of invasion showed the strongest correlation with loss of E-cadherin expression. On the other hand, no association of E-cadherin was found with WHO histological grading, degree of keratinization, number of mitoses, lymphoplasmacytic response and lymph node metastasis.

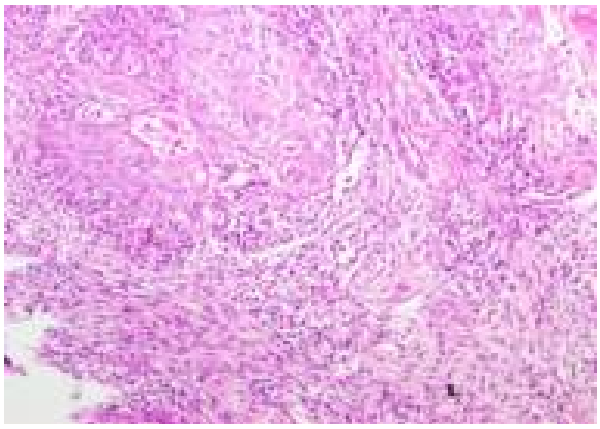

Figure 1. Photomicrograph showing pattern of invasion of OSCC: Score 1 (Pushing, well-delineatedinfiltrating borders) (H&E, X 200)

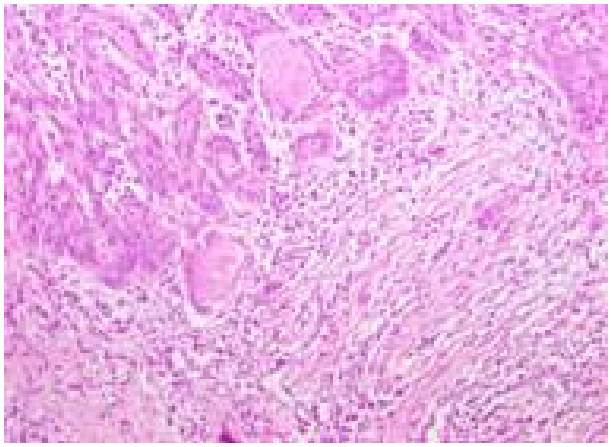

Figure 2. Photomicrograph showing pattern of invasion of OSCC: Score 2 (Infiltrating, solidcords, bands and/orstrands) (H&E, X 200)

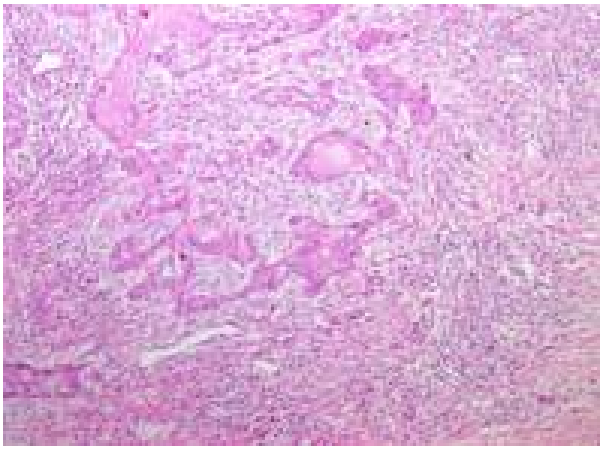

Figure 3. Photomicrograph showing pattern of invasionof OSCC: Score 3 (Small groups or cordsof infiltrating cells (n>15) (H&E X200)

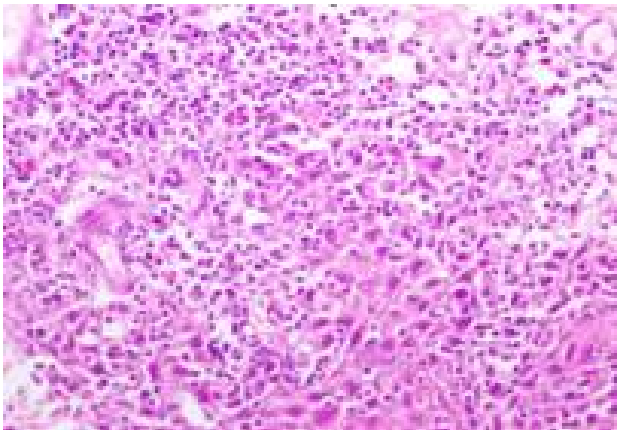

Figure 4. Photomicrograph showing pattern of invasion of OSCC: Score 4 (Marked and wide-spread cellulardissemination in small groups and/or in single cells (n<15) (H&E X 400)

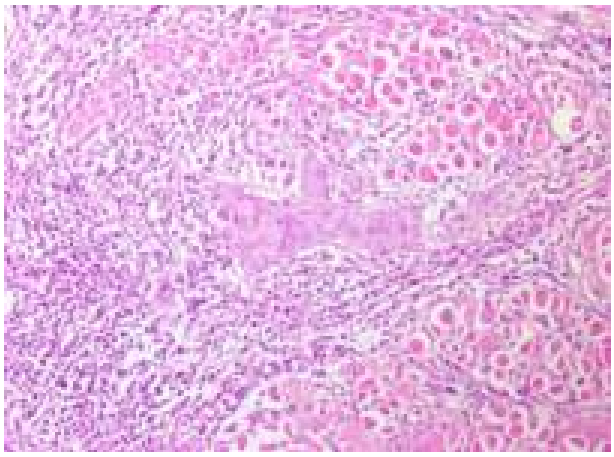

Figure 5. Photomicrograph showing stage of invasion of OSCC: Score 3 (Invasion below laminapropria adjacent tomuscles) (H&E X 200)

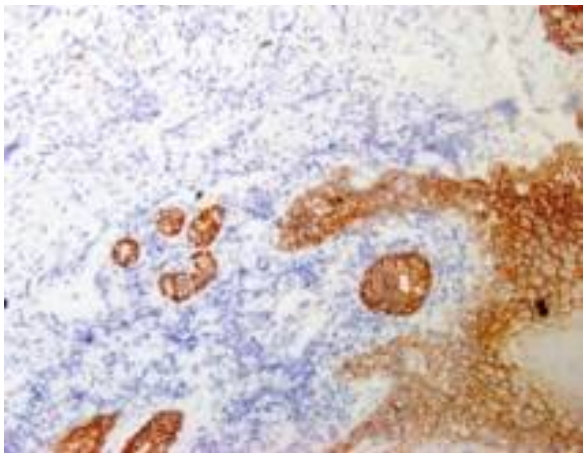

Figure 7. Photomicrograph of a well differentiated OSCC with positive membranous staining of E-cadherin: Score 7 (IHC X 200)

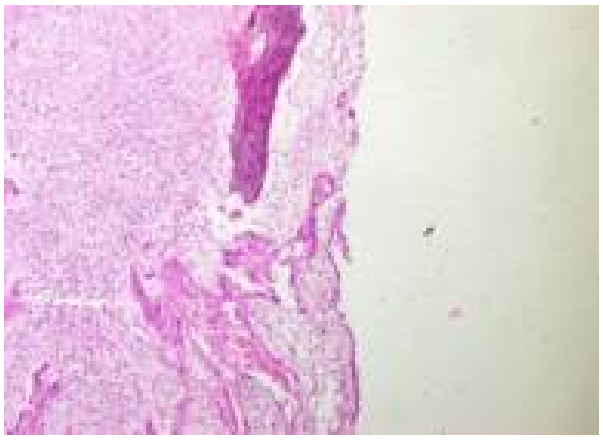

Figure 6. Photomicrograph showing stage of invasion of OSCC: Score 4 (Extensive and deepinvasion replacingmost of the stromaltissue and infiltrating jaw bone) (H&E X 100)

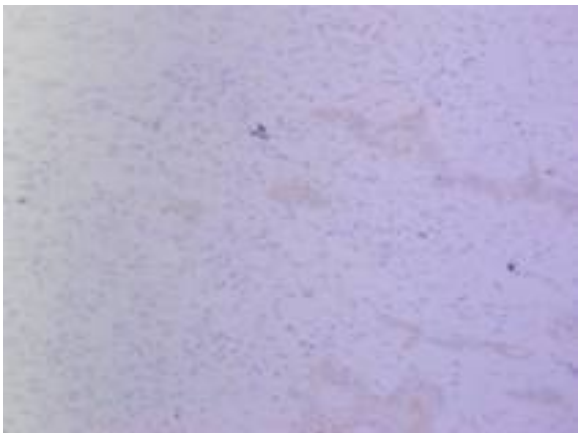

Figure 8. Photomicrograph of a moderately differentiated OSCC with negative membranous staining of E-cadherin: Score 3 (IHC X 200)

Table II: Correlation of E-cadherin expression with histological prognostic parameters of oral squamous cell carcinoma

Histomorphological parameters		No. of cases	E-cadherin immunoreactivity		p-value
			Positive	Negative	
WHO histological grade	Grade I	20	10 (50%)	10 (50%)	0.44
	Grade II	29	14 (48.3%)	15 (51.7%)	
	Grade III	12	5 (41.7%)	7 (58.3%)	
Degree of keratinization	Score 1	12	7 (58.3%)	5 (41.7%)	0.12
	Score 2	19	11 (57.9%)	8 (42.1%)	
	Score 3	16	4 (25%)	12 (75%)	
	Score 4	14	7 (50%)	7 (50%)	
Nuclear pleomorphism	Score 1	4	4 (100%)	0 (0%)	0.001*
	Score 2	29	16 (55.2%)	13 (44.8%)	
	Score 3	15	6 (40%)	9 (60%)	
	Score 4	13	3 (23.1%)	10 (76.9%)	
Number of mitoses	Score 1	9	6 (66.7%)	3 (33.3%)	0.44
	Score 2	35	16 (45.7%)	19 (54.3%)	
	Score 3	13	4 (30.8%)	9 (69.2%)	
	Score 4	4	3 (7550	1 (25%)	
Pattern of invasion	Score 1	6	5 (83.3%)	1 (16.7%)	<0.001**
	Score 2	28	19 (67.9%)	9 (32.1%)	
	Score 3	9	3 (33.3%)	6 (66.7%)	
	Score 4	18	2 (11.1%)	16 (88.9%)	
Stage of invasion	Score 1	0	0 (0%)	0 (0%)	0.001*
	Score 2	8	4 (50%)	4 (50%)	
	Score 3	21	11 (52.4%)	10 (47.6%)	
	Score 4	7	0 (0%)	7 (100%)	
Lymphoplasmacytic response	Score 1	12	5 (41.7%)	7 (58.3%)	0.878
	Score 2	22	11 (50%)	11 (50%)	
	Score 3	23	9 (39.1%)	14 (60.9%)	
	Score 4	4	4 (100%)	0 (0%)	
Anneroth's total grade	Grade I	8	5 (62.5%)	3 (37.5%)	0.019 *
	Grade II	21	8 (38.1%)	13 (61.9%)	
	Grade III	7	2 (28.6%)	5 (71.4%)	
Lymph node metastasis	Present	8	4 (50%)	4 (50%)	0.864
	Absent	13	7 (54%)	6 (46%)	

*Significant at <0.05

Discussion

Several previous studies showed that many clinicopathological parameters and E-cadherin expression had been asssociated with local recurrence or death in oral squamous cell carcinoma patients.[1,17,18] Most of the previous studies assessed histological grade, TNM stage, pattern of invasion and lymph node metastasis as prognostic parameters.[1,12,19] Some of these parameters were found to have an association with loss of E-cadherin and some were not.[1,19] In addition to WHO (1971) histological grade and lymph node metastasis, each of the six histomorphological parameters of Anneroth grading were evaluated in this study in association with expression of E-cadherin. Anneroth's described grading system had

been proved previously by several independent workers to have better prognostic value over WHO (1971) histological grading.[20,21,22] Conventional (1925)[3]/WHO grading system has lesser prognostic value as because this system focuses mainly on the degree of keratinization. Other important parameters such as invasive pattern is not considered here. Chance of interobserver variability is more in this system whereas the parameters of Anneroth grading system are more reproducible.[6] Here in this study, each of the six parameters of Anneroth were analyzed in excised tumor samples in association with E-cadherin expression to assess the significance of each of these factors appropriately. Crissman[5] et alnoted that a single component might have a more clinical value over the combined score of multiple histologic parameters.

This study found no significant correlation between E-cadherin expression and WHO (1971) histological grading. Liu et al[1] in 2010 examined 83 oral squamous cell carcinoma cases and found no association of E-cadherin expression with WHO (1971) or conventional Broder grading. Similarly, Mehendirattaetal[17] evaluated the invasive front of thirty cases and they also did not demonstrate any association of E-cadherin expression with WHO (1971) histological grading. Although some other studies established significant association of loss of E-cadherin expression with histological grading.[23,24]
Degree of keratinization, number of mitoses and lymphoplasmacytic response were also found to have no association with E-cadherin expression in this study. Degree of keratinization in squamous cell carcinoma was found to have less value in predicting patient survival or lymph node metastasis in a previous study.[5] In 1984, Crissmanet al[5] evaluated multiple histological parameters in seventy seven cases of oral cancer and found no significant association of degree of keratinization, nuclear pleomorphism, number of mitoses or inflammatory response with patient outcome. Similarly, Odell et al[20] also analyzed multiple histological parameters from 106 cases of oral squamous cell carcinoma in 1994. They found no association of nuclear pleomorphism or host response with recurrence or lymph node metastasis.[20] But they stated that local recurrence or metastasis were significantly correlated with Broder's grade and degree of keratinization. According to this study, the correlation of nuclear pleomorphism with loss of E-cadherin expression was statistically significant.

Pattern of invasion is the parameter which was most frequently studied in oral squamous cell carcinoma patients in relation to prognosis or patient outcome and also in association with prognostic markers including E-cadherin. This is one of the most important prognostic parameter of Anneroth grading system. According to Crissmanet al,[5] pattern of invasion was the single most important histologic variable in predicting patient survival. Odell et al[20] also demonstrated pattern of invasion as a significant prognostic parameter in their study. The significance of pattern of invasion as a prognostic indicator was enhanced because it was the most accurately scored parameter among observers and the scores were relatively evenly distributed among tumors.[6,25] The current study demonstrated that the frequency of E-cadherin expression in relation to pattern of invasion were 83.3%, 67.9%, 33.3% and 11.1% in score 1, score 2, score 3 and score 4 cases respectively. The correlation of pattern of invasion with loss E-cadherin expression was statistically significant and was the strongest among all parameters examined here. This finding is consistent with most of the previous studies.[19,26,27] In contrast, Liu et al[1] in their study found no association of E-cadherin expression with pattern of invasion.

Stage or depth of invasion is another important component of Anneroth's multifactorial grading system.[4] Several independent workers established depth or stage of invasion as an important prognostic indicator. Most of them used a cut-off value for tumor thickness and observed a significant association between increasing tumor thickness and worse patient outcome.[28,29] Moore et al[30] noted that tumor thickness was an accurate predictor of lymph node metastasis. Although there are several studies regarding TNM staging or clinical staging in association with E-cadherin expression, no significant data were found about histological staging or depth of invasion in association with expression of E-cadherin.[1,17,31] Usefulness of this parameter might be limited becauseit requires total resection of the lesion in order to be evaluated. In the present study, stage of invasion was examined in only 36 cases excluding oralmucosal biopsy specimens and found a significant correlation between stage of invasion and loss of E-cadherin expression.

After summation of the scores for each of the six histological parameters, total Anneroth grading was also done in 36 cases which fulfilled the criteria for all of these parameters to be examined. A significant association between increasing Anneroth grade and loss of E-cadherin expression was found in this study. The prognostic value of this grading system over Broders' or WHO(1971) histological grading system was already established by several independent workers which was discussed previously.[21,25,32]

Out of total 61 cases, only 21 specimens included lymph nodes with the excised tumor. Lymph node status were evaluated in these cases only and there were no significant association of expression of E-cadherin with lymph node metastasis. This supports the result of a previous study done by Liu et al,[1] who also did not find any association of E-cadherin expression with lymph node metastasis. But there are many other studies which demonstrated significant association of E-cadherin expression with lymph node metastasis.[17,23,33] The reason behind this dissimilarity might be due to small sample size, variable expression of E-cadherin in the tumor cells at different invasive fronts of the same tumor or because different scoring system used by different workers for evaluation of the positivity of this marker. It would be more conclusive if a prospective study could be done with larger number of excised tumor including lymph nodes and long term follow-up data.

Conclusion

The overall findings of these study reflect that Anneroth grading system especially the parameter pattern of invasion has more prognostic value over WHO (1971) histological grading system. If this grading system is used in addition to conventional grading in case of evaluation of OSCC cases, it will be more beneficial for predicting patient outcome as well as planning of further management. E-cadherin immunohistochemistry is also very much helpful in predicting tumor behavior, prognosis and can serve as a molecular target for therapeutic implementations.

However, larger studies are recommended in future for these purposes to be fulfilled. Large sample size including lymph nodes with long term follow-up data and a consistent scoring system for evaluation of E-cadherin expression are needed to have a more conclusive result.

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