ORIGINAL ARTICLE

Correlation of modified Anneroth's histological grading system with E-cadherin expression in squamous cell carcinoma of oral cavity and oropharynx

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Abstract

Background: E-cadherin, a cell-cell adhesion molecule, is an "Invasion suppressor." Loss of E-cadherin expression is associated with loss of epithelial differentiation and acquisition of metastatic potential in cancers of epithelial origin.

Objectives: The objectives of the study were to evaluate the correlation between the E-cadherin immunoreactivity score (IRS) and modified Anneroth's histological parameters and grade groups of squamous cell carcinoma (SCC) of oral cavity (OC) and oropharynx (OP).

Materials and Methods: Histomorphological grading was done using modified Anneroth's grading system in 50 cases of SCC of OC and OP and tumors were categorized into three groups. E-cadherin immunomarker was evaluated for its proportion and intensity of expression in the tumor cells and the scores were correlated with histological scores by Pearson's correlation coefficient (r).

Results: Histological parameters such as reduced keratinization, higher grade of nuclear pleomorphism, and worst pattern of invasion (WPOI) were found to be strongly associated with reduced E-cadherin expression. Overall, with worsening histological grade groups, the E-cadherin expression in the tumor was seen declining ($P = 0.0000063$). The histological scores were inversely related to E-cadherin IRS ($r = -0.83229$).

Conclusion: The study found that WPOI, nuclear pleomorphism, and degree of keratinization are important histological parameters in grading the SCC in incisional biopsies. However, the prognostic significance could not be elucidated in our study. We found that with the worsening of above parameters and histological grade of the tumor, the E-cadherin expression was reduced indicating the loss of epithelial phenotype in the tumor cells of higher grade.

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide.[1-3] In spite of the modifications in the management, the 5-year survival remains less than 50% because of recurrence and distant spread.[4]

Histological grading of squamous cell carcinoma (SCC) of oral cavity (OC) and oropharynx (OP) is the important predictor of tumor behavior. Broder's grading is being used since decades to grade the SCC at different locations. As it lacks reliability and prognostic significance, a search for better grading system is demanded which led to the application of multiparametric modified Anneroth's grading system in the present study.[5-7]

Worsening histological grade of the tumor is associated with loss of epithelial differentiation and acquisition of mesenchymal phenotype. This epithelial mesenchymal transition is characterized by loss of cell-cell adhesion.[8] E-cadherin, a cell-cell adhesion molecule encoded by CDH1 gene located on chromosome 16q21, plays an important role in maintaining the normal architecture and cell polarity. Loss of this molecule in tumor cell is associated with greater invasive potential. It also helps to predict more aggressive tumors and aid in clinical decision-making, therapeutic strategies, and ultimately patients' outcome.[9] The present study was conducted to evaluate the correlation between the E-cadherin immunoreactivity score (IRS) and modified

Anneroth's histological parameters and grade groups of SCCs of OC and OP.

Materials and Methods

The study included 50 cases of SCC of OC and OP from June 2017 to May 2020. The samples included incisional biopsies, excisional biopsies, and radical resected specimens. Most of the specimens were incisional biopsies constituting 86% (n = 42) as majority were referred to the higher centers for further management. Incisional biopsies of adequate size not less than 0.5 cm and with the presence of tumor host population were included in the study. Specimens were processed using Leica Automated Vacuum tissue processor ASP6025, blocks made were cut into 3 μm thick sections and stained with hematoxylin and eosin. Histological grading of the tumor was done using modified Anneroth's histological grading system [Table 1].[6,7]

Tumors were categorized into three groups based on the scores obtained after summation of the scores of parameters used in the grading system as score 5–10 = Group I; score 11–15 = Group II; and score 16–20 = Group III.[6,7]

Histologically confirmed cases of SCC of OC and OP tissue sections were stained for E-cadherin immunohistochemical marker using heat-induced antigen retrieval, hydrogen peroxide blockade of endogenous peroxidase, and incubation of tissue sections with E-cadherin monoclonal antibody EP6 clone, Biocare medical, USA (Ready to use, Dilution 1:50). Visualization was performed using DAB (diaminobenzidine tetrahydrochloride) as chromogen and hematoxylin as counterstain. Control slides were run concurrently with the study slides following the same procedure.

E-cadherin expression was quantified for its proportion and intensity of staining in tumor population. The proportion of tumor cells positive for membranous E-cadherin was scored as zero = no detectable expression; 1+ = expression in <10% tumor cells; 2+= expression in 10–50% cells; 3+ = expression in 51–80% cells; and 4+ = expression in >80% cells.[9] The intensity of E-cadherin expression was also scored as zero = absent staining; 1+ = weak staining; 2+ = moderate staining; and 3+ = strong staining.[10]

The IRS was obtained by multiplying the proportion of tumor cells positive for membranous E-cadherin and intensity of staining. The IRS was grouped into four categories as follows – score 0, 1 = negative; score 2–3 = low; score 4–8 = high; and score 9–12 = very high.[10] The E-cadherin IRS was compared with each of the parameters and overall grade of the tumor. The correlation between E-cadherin IRS and histological score of the tumor was studied.

Statistical analysis

Data were entered into MS Excel and analyzed using data analysis software. Categorical and nominal variables were compared using Chi-square test. $P < 0.05$ was considered as statistical significance. The relation between the histological score and E-cadherin IRS was done using Pearson's correlation coefficient test. The value of correlation coefficient (r) was used to denote the strength of correlation.

Results

Among 50 cases, 42 cases were incisional biopsies, three excision biopsies, and five radical resections. The radical resections included three segmental mandibulectomy, one bite resection, and one glossectomy specimen.

The age of the patients ranged from 33 years to 83 years with mean age of 54.5 ± 12.5 years. Of 50 cases, 43 were male and only 7 cases were female with male-to-female ratio of 6.14:1. Majority of the cases were located in tongue and buccal mucosa accounting to 38% and 28%, respectively.

Group I histological grade SCC cases showed high to moderately keratinized tumor cells showing little to moderate nuclear pleomorphism with 0–1 mitotic figure/HPF [Figure 1a]. Majority of the cases showed pushing well-delineated infiltrating borders as predominant worst pattern of invasion (WPOI). Two

Table 1: Modified Anneroth's histological grading system

Morphological parameters	Score 1	Score 2	Score 3	Score 4
Histological grading of malignancy of tumor cell population				
Degree of keratinization	Highly keratinized (>50% cells)	Moderately keratinized (20–50% cells)	Minimal keratinization (5–20% cells)	No keratinization (<5% cells)
Nuclear pleomorphism	Little nuclear pleomorphism (75% mature cells)	Moderate nuclear pleomorphism (50–75% mature cells)	Abundant nuclear pleomorphism (25–50% mature cells)	Extreme nuclear pleomorphism (0–25% mature cells)
Number of mitosis (/hpf)	0–1/hpf	2–3/hpf	4–5/hpf	>5/hpf
Histological grading of malignancy of tumor-host population				
WPOI	Pushing well-delineated infiltrating borders (WPOI-1)	Infiltrating solid cords, bands, and strands (WPOI-2)	Small groups or cords of more than 15 cells (WPOI-3)	Marked and widespread cellular dissociation and single-cell infiltration (WPOI-4)
Lymphoplasmacytic infiltration	Marked	Moderate	Slight	None

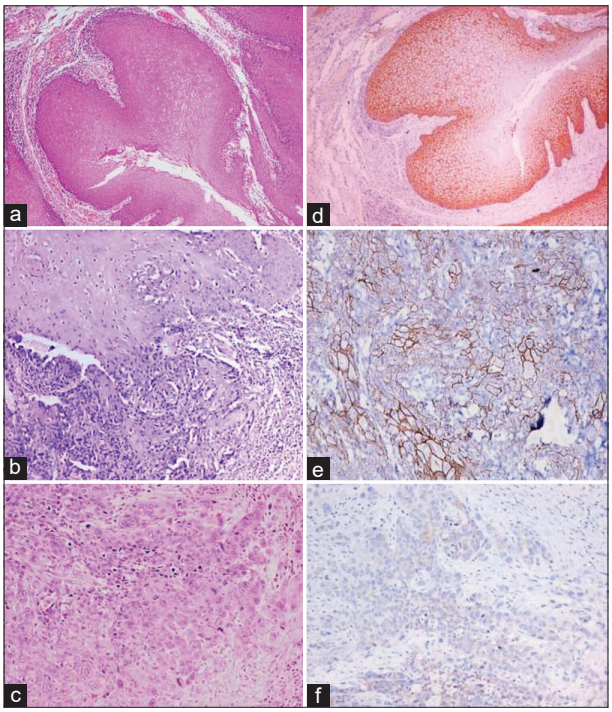

Figure 1: (a) Grade Group I squamous cell carcinoma (SCC) with modified Anneroth's histological score = 9 (hematoxylin and eosin [H&E], 200×). (b) Grade Group II SCC with modified Anneroth's histological score = 14 (H&E, 400×). (c) Grade Group III SCC with modified Anneroth's histological score = 19) (H&E, 400×). (d) Grade Group I SCC showing very high (immunoreactivity score [IRS] = 9) E-cadherin expression (IHC, 200×), (e) Grade Group II SCC showing low (IRS = 4) E-cadherin expression (IHC, 400×). (f) Grade Group III SCC showing negative (IRS = 0) E-cadherin expression (IHC, 400×)

cases showed moderate lymphoplasmacytic infiltration and one case each showed marked and slight infiltration of lymphocytes and plasma cells. However, the p value for histological parameters used to grade the tumor was not statistically significant ($P = 0.401$) [Table 2].

Group II histological grade tumors predominantly showed minimal keratinized cells ($n = 14$) with majority showing moderate nuclear pleomorphism and 2–3 mitotic figures/HPF [Figure 1b]. The WPOI varied from WPOI-2 to WPOI-4. Majority showed ($n = 10$) moderate infiltration of lymphocytes and plasma cells. The p value was statistically significant ($P = 0.00078$) [Table 2].

Group III histological grade tumors showed 8 cases with <5% cells with keratinization, majority showed extreme nuclear pleomorphism with mitotic figures ranging from 0–1/HPF to one case with >5 mitotic figures/HPF [Figure 1c]. Cases showed WPOI-3 and WPOI-4. Moreover, lymphoplasmacytic infiltration was mild in majority of the cases. p value was statistically significant ($P = 0.00069$) [Table 2].

E-cadherin expression of the tumor was compared with different parameters of modified Anneroth's histological grading system and found that reduced keratinization, higher degree of nuclear pleomorphism, and WPOI in the tumor were strongly associated with reduced E-cadherin expression [Figure 1d-f]. Whereas, mitotic activity and lymphoplasmacytic infiltration were found to have no significant association with E-cadherin expression in tumor cells [Table 3].

Furthermore, the comparison of modified Anneroth's histological grade groups with E-cadherin expression showed that the higher histological grade tumors were associated with lower E-cadherin expression which was statistically significant ($P = 0.0000063$). The Pearson's correlation coefficient (r) was −0.83229 [Table 3].

Discussion

Histological grading of the tumor has been used since many decades in an attempt to predict the clinical behavior of SCC of OC and OP. Due to lack of correlation in prognostic significance of Broder's grading system, a lot of other systems have modified it by inclusion of multiple parameters that helped to obtain a more precise morphologic evaluation of the SCCs in the head-and-neck region.[11-13]

Anneroth and Hansen modified the Jakobson's system of grading,[12] a modification of Broder's system, by omission of vascular invasion of the tumor used in the former system. The Anneroth's system was tested in 89 cases of SCC of floor of the mouth and found that total histological scores were strongly associated with clinical staging, frequency of recurrence, and death from primary carcinoma of OC. Studies have also found that Anneroth's system of grading is superior to Broder's grading and more reliable multiparametric prognostic tool in assessing the behavior of the tumor.[5,6] In the present study, modification of the Anneroth's grading system was used by omitting the depth of invasion (DOI) from the grading system as most of our cases were incisional biopsies where the DOI could not be determined.

In the present study of 50 cases, most of the cases were clustered between 41 and 70 years with mean age of 54.5 years. The study had male preponderance which is similar to other studies.[14-16] Tongue was the most common primary site of OSCC as in other studies.[17-19]

Higher histological grade tumors showed reduced keratinization, greater nuclear pleomorphism, pattern 3 and pattern 4 of invasion, increased mitotic activity, and reduced host immunological response. However, these parameters did not have significant association with grade Group I tumors. This could be because of smaller sample size in that group.

The deteriorating degree of keratinization, nuclear pleomorphism, and POI were found to have strong association with declining E-cadherin expression in our study. It is determined in other studies that POI is the single most important prognostic parameter in predicting the behavior of SCC of OC and OP[20,21] The adverse POI was strongly associated with reduced E-cadherin expression in other studies as well.[22-24] The tumors with higher degree of nuclear pleomorphism showed

Table 2: Modified Anneroth's histological grading of the cases

	Degree of keratinization (No. of cases)	Nuclear pleomorphism (No. of cases)	No. of mitosis (No. of cases)	WPOI (No. of cases)	Lymphoplasmacytic infiltration (No. of cases)	*P* value
Group I : n=4						0.401
Score 1	2	3	4	3	1	
Score 2	2	1	0	1	2	
Score 3	0	0	0	0	1	
Score 4	0	0	0	0	0	
Group II : n=24						0.00078
Score 1	1	3	9	0	8	
Score 2	8	14	13	13	10	
Score 3	14	7	2	9	6	
Score 4	1	0	0	2	0	
Group III : n=22						0.00069
Score 1	0	0	2	0	4	
Score 2	7	6	10	0	6	
Score 3	7	7	9	11	10	
Score 4	8	9	1	11	2	

Table 3: Comparison of E-cadherin expression with different parameters and grade groups of modified Anneroth's histological grading system

Parameters	No. of cases	E-cadherin IRS (No. of cases)				*P* value
		0–1	2–3	4–8	9–12	
Degree of keratinization						
Marked (>50%)	2	0	0	1	1	0.000372
Moderate (20–50%)	18	0	4	11	3	
Minimal (5–20%)	21	2	1	17	1	
Nil (<5%)	9	8	0	0	1	
Nuclear pleomorphism						
Mild	6	0	0	3	3	
Moderate	21	1	1	18	1	0.000222
Marked	14	4	1	7	2	
Extreme	9	5	3	1	0	
No. of mitosis						
0–1/hpf	15	1	2	8	4	
2–3/hpf	23	4	1	17	1	0.11
4–5/hpf	11	4	2	4	1	
>5/hpf	1	1	0	0	0	
WPOI						
WPOI-1	3	0	0	0	3	
WPOI-2	14	0	2	10	2	0.0000496
WPOI-3	20	5	1	14	0	
WPOI-4	13	4	4	4	1	
Lymphoplasmacytic infiltration						
Marked	13	1	1	9	2	
Moderate	18	2	4	10	2	
Mild	17	6	1	8	2	0.517918
NIL	2	1	0	1	0	
Modified Anneroth's histological grade groups						
Group I (score=5–10)	4	0	0	1	3	
Group II (score=11–15)	24	0	2	19	3	0.0000063
Group III (score=16–20)	22	9	5	8	0	

Pearson's correlation coefficient (r) = −0.83229

reduced E-cadherin expression but the degree of keratinization had no significant association with E-cadherin expression in one of the studies.[24]

In our study, mitotic activity and host immune response did not have association with E-cadherin expression which was similar to a retrospective study done in 61 cases.[24]

The overall histological score of the tumor when correlated with E-cadherin IRS was found to have a strong negative "*r*" value of −0.83229, indicating that with higher histological grade of the tumor, the E-cadherin expression in the tumor cells was reduced as observed in other studies.[19,24-27]

We could not determine DOI, lymphovascular invasion (LVI), and perineural invasion (PNI) as majority of our cases were incisional biopsies. Furthermore, the tumor budding is mentioned as the worst POI-5 in the reporting of SCC of OC and OP but could not be determined in the incisional biopsies which were in greater number.

Among the five radical resected cases, only two cases had lymph node metastasis (LNM) which was very insignificant to compare and to draw a conclusion. Greater sample size with a greater number of resected specimens would give better view of comparison of the different histomorphological prognostic parameters with E-cadherin expression in the tumor.

Although incisional biopsies were in major proportion, in contrast to other studies where radical resections of the tumors were more, the inverse correlation of E-cadherin expression and histological grade of the tumor did not vary when compared with others.[15,17,24]

Despite the limitations of our study, the combination of modified Anneroth's histological grading of the tumor and prognostic immunological markers like E-cadherin in small biopsies could still help in understanding the characteristics of the tumor and its behavior with respect to its morphology and relationship with the host environment and to be applied while histopathological reporting.

Conclusion

SCC of OC and OP is one of the most common cancers and accounts to high mortality and morbidity. The present study found a strong inverse correlation between histological grade of the tumor and E-cadherin expression. Among the histological parameters used to grade the tumor, degree of keratinization, nuclear pleomorphism, and POI showed strong association with E-cadherin expression. However, correlation with DOI, LVI, PNI, LNM, and clinical staging is needed for more conclusive results regarding prognostic importance of Anneroth's histological grading system and E-cadherin immunomarker in SCC of OC and OP.

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