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Association of Vimentin Expression with Various Histopathological Parameters According to WHO and Anneroth Grade in Oral Squamous Cell Carcinoma

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Abstract

Background: *Numerous factors that increase the risk of oral cancer have been linked to its development. Therefore, there is a pressing need to discover new molecular indicators that can predict the behavior of the cancer and potential targets for treatment. Vimentin has shown a strong correlation with the advancement of oral squamous cell carcinoma (OSCC). This study aims to identify dependable histologic prognostic factors and investigate the association in between vimentin expression and these factors according to Anneroth and WHO grade in OSCC.*

Objective: *Association of vimentin expression with Anneroth and WHO grade of OSSC.*

Method: *A cross-sectional study was carried out in the Department of Pathology, Shaheed Suhrawardy Medical College and Hospital, Dhaka, Bangladesh from September 2021 to August 2023. A total of 51 histologically diagnosed cases of OSCC were selected for the study. Among these, 25 cases were resected tumor specimens and 26 were oral mucosal biopsy. Paraffin blocks and slides were prepared after grossing. H&E and IHC staining were done and analyzed accordingly. All cases were categorized into groups according to WHO and Anneroth grading. The statistical analysis was carried out using the SPSS version 26.*

Result: *In this study, a significant association of high vimentin expression was found with degree of keratinization, nuclear pleomorphism, pattern of invasion, and stage of invasion. which means that there is a significant association of high vimentin expression with worsening of these parameters. On the other hand, no association of vimentin was found with number of mitoses and lymphoplasmacytic responses. Association of WHO histological grade with Vimentin immunoreactivity was statistically significant. Association between total Anneroth grade and Vimentin immunoreactivity was statistically significant also.*

Discussion: *Based on the findings, the individual histopathological prognostic parameters can be used for predicting patient outcomes. If these parameters are used in addition to conventional grading in the case of evaluation of OSCC cases, could be more beneficial for predicting patient outcomes as well as planning further management.*

Keywords: Oscc, Vimentin, H&E, IHC, WHO, Anneroth.

Introduction

Squamous cell carcinoma preferentially develops on the ventral surface of the tongue, the floor of the mouth, the lower lip, the soft palate, and the gingiva. Oral cancer is the sixth most common type of cancer worldwide. With a five-year survival rate of roughly 45%, oral cancers are still challenging to diagnose and treat despite advancements in diagnostic and therapeutic techniques. 2020, there were 8137 fatalities and 13985 (8.9%) new cases in Bangladesh (Globocan, 2020).The prognosis for oral squamous cell carcinoma is often poor due to local recurrence and lymph node metastases [1-6].

Vimentin is a cytoskeleton-related protein that weighs 57 kDa. Cell morphological changes, a decrease in cell-cell contact, and an increase in cell motility are all consequences of vimentin overexpression in epithelial cells, causing tissue invasion and metastasis to distant organs [7]. Vimentin also controls lymph node cell migration. Therefore, vimentin expression increase is strongly linked to the invasion, metastasis, and differentiation of cancer cells.

Broder's (1925)/ Who Histological Grading (1971) System

According to this system, tumors are graded into three groups:

- Well-differentiated or grade I: composed of relatively mature tumor cells with few nuclear aberrations. These tumors show presence of keratin pearls and/or individual cell keratinization.
- Moderately differentiated or grade II: Keratinization is occasionally present and nuclear aberrations are moderately abundant
- Poorly differentiated or grade III: show no tendency towards keratinization. Nuclear aberrations are abundant

Anneroth's Grading System (1987)

The histologic grading of malignancy is made using six morphological criteria:

- Tendency to keratinization
- Nuclear aberrations or pleomorphism
- Number of mitoses
- Pattern of invasion
- Stage of invasion
- Lymphohistiocytic/inflammatory response

Each of the parameters are scored as 1 to 4 points based on microscopic findings.

The purpose of this study is to observe vimentin immunostaining of paraffin-embedded oral cancer tissue in biopsy/resected specimens and evaluate its association with various histopathological parameters like degree of keratinization, nuclear pleomorphism, number of mitoses, pattern of invasion, lymphoplasmacytic response, lymphnode metastasis (Anneroth grading system) in order to determine its predictive value for invasion and metastasis of oral squamous cell carcinoma.

Materials and Methods

A cross-sectional study was carried out in the Department of Pathology, Shaheed Suhrawardy Medical College Hospital, Dhaka, Bangladesh from September 2021 to August 2023. A total of 51 histologically diagnosed cases of OSCC were selected for the study. Among these, 25 cases were resected tumor specimens and 26 were oral mucosal biopsy. Paraffin blocks and slides were prepared after grossing. H&E and IHC staining were done and analyzed accordingly. Then, all cases were categorized into well, moderate and poorly differentiated groups according to WHO defined criteria.

All of the six individual morphological parameters of Anneroth's grading system could be evaluated in 25 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 rest of the 26 oral mucosal biopsy specimens. In these cases, rest of the five morphological parameters were evaluated. After summation of the separate scoring points, these cases were also classified into grade I (6-12), II (13-18) and III (19-24).

Regarding the IHC Staining Intensity, Cases were Analyzed Under Light Microscopy (40X), Using the Following 3 -Point Scoring System:

0 (Negative)
1+ (Weak)
2+ (Moderate)
3+ (Strong)

According to the Percentage of Positive cells, Cases were Scored as:

0 = Negative
1 = <10%
2 = 11–50%
3 = 51–80%
4 = >80%

The Final Score Calculated by Multiplying staining intensity and positivity in tumor cells (range 0–12) dividing into three groups:

0= Negative
1-4= Low expression
> 4 = High expression9

The statistical analysis was carried out using the SPSS version 26.

Result

Table 1: Distribution of the Patients with OSCC According to WHO and Anneroth Grading System Majority of the Cases Belong to Grade 1 in Both WHO and Anneroth Grading.

	No. of cases	
	WHO Grade	Anneroth Grade
Grade		
Grade I	30(58%)	14(56%)
Grade II	12(23%)	7(28%)
Grade III	9(17%)	4(16%)
Total	51	25

Table 2: Association between WHO histological grade and Vimentin immunoreactivity (n=51) Among the 51 cases, Vimentin high expression were 37%, 67%, and 89% for the cases having grade 1, 2, and 3 respectively. Chi-Square (X2) test p-value (0.012) is Statistically Significant.

	No. of cases	**Vimentin**		**p-value**
		High	**Low**	
WHO grade				
Grade I	30	11 (37%)	19 (63%)	
Grade II	12	8 (67%)	4 (33%)	
Grade III	9	8 (89%)	1 (11%)	
Total	51	27 (53%)	24(47%)	0.012[s]

Table 3: Association between degree of keratinization and Vimentin Immunoreactivity (n = 51) Among the 51 cases, Vimentin high expression were 30%, 63%, 100% and 75% for the cases having score 1, 2, 3 and 4 respectively. Chi-Square (X2) test p-value is 0.013 that is Statistically Significant.

	No. of cases	Vimentin		p-value
		High	Low	
Degree of keratinization				
Score 1	23	7 (30%)	16(70%)	
Score 2	19	12 (63%)	7 (37%)	
Score 3	5	5 (100%)	0 (0%)	
Score 4	4	3 (75%)	1 (25%)	
Total	51	27 (53%)	24(47%)	0.013[s]

Table 4: Association of Nuclear Pleomorphism and Vimentin Immunoreactivity (n = 51) Among the 51 cases, Vimentin high expression were 20%, 45%, 83% and 86% for the cases having score 1, 2, 3 and 4 respectively. Chi-Square (X2) test p-value is 0.043, which is Statistically Significant.

	No. of cases	**Vimentin**		**p-value**
		High	**Low**	
Nuclear pleomorphism				
Score 1	5	1 (20%)	4 (80%)	0.043[s]
Score 2	33	15 (45%)	18 (55%)	
Score 3	6	5 (83%)	1 (17%)	
Score 4	7	6 (86%)	1 (14%)	

Table 5: Association between Number of Mitoses and Vimentin Immunoreactivity (n=51) Among the 51 cases, Vimentin High Expression were 33% for score 1 cases, 46.0% for score 2 cases, 89% for score 3 cases, and 75.0% for score 4 cases. Chi-Square (X2) test p-value is 0.051 which is not Statistically Significant.

	No. of cases	**Vimentin**		**p-value**
		High	**Low**	
Number of mitoses				

Score 1	12	4 (33%)	8 (67%)	0.051n^{s}
Score 2	26	12 (46%)	14 (54%)	
Score 3	9	8 (89%)	1 (11%)	
Score 4	4	3 (75%)	1 (25%)	

Table 6: Association between Pattern of Invasion and Vimentin Immunoreactivity (n=51) 50% of the score 1 cases, 37% of the score 2 cases, 75% of the score 3 cases and 86% of the score 4 cases showed high immunoreactivity for Vimentin. Chi-Square (X2) test p-Value is 0.035, which is Statistically Significant.

	No. of cases	Vimentin		p-value
		High	Low	
Pattern of invasion				
Score 1	2	1 (50%)	1 (50%)	0.035^{s}
Score 2	30	11 (37%)	19 (63%)	
Score 3	12	9 (75%)	3 (25%)	
Score 4	7	6 (86%)	1 (14%)	

Table 7: Association between Stage of Invasion and Vimentin Immunoreactivity (n=25) Among the 25 cases, Vimentin High Expression were 13% for score 2 cases, 71% for Score 3 Cases and 67% for Score 4 Cases. Chi-Square (X2) test p-Value is 0.025, Which is Statistically Significant.

	No. of cases	Vimentin		p-value
		High	Low	
Stage of Invasion				
Score 2	8	1 (13%)	7 (88%)	0.025^{s}
Score 3	14	10 (71%)	4 (29%)	
Score 4	3	2 (67%)	1 (33%)	

Table 8: Association between Lymphoplasmacytic Response and Vimentin Immunoreactivity (n=51) Among the 51 Cases, Vimentin High Expression were 54% for score 1 cases, 51% for score 2 cases 45% for score 3 cases and 100% for score 4 cases. Chi-Square (X2) test p-Value is 0.056, which is not Statistically Significant.

	No. of cases	Vimentin		p-value
		High	Low	
Lymphoplasmacytic Re-sponse				
Score 1	13	7 (54%)	6 (46%)	0.056^{ns}
Score 2	25	13 (51%)	12 (48%)	
Score 3	11	5 (45%)	6 (55%)	
Score 4	2	2 (100%)	0 (0%)	

Table 9: Association between Total Anneroth Grade and Vimentin Immunoreactivity (n=25) Total Anneroth Grade was Calculated in 25 Cases after Summation of the Scores for the Six Histomorphological Parameters Described Above. 29% of the total 14 Grade I Cases, 86% Grade II Cases and 3 75% of the Grade III Cases Showed Vimentin High Expression. Chi-Square (X2) test p-value is 0.029, which is Statistically Significant.

	No. of cases	Vimentin		p-value
		High	Low	
Anneroth's total grade				
Grade 1	14	4 (29%)	10 (71%)	0.029s
Grade 2	7	6 (86%)	1 (14%)	
Grade 3	4	3 (75%)	1 (25%)	

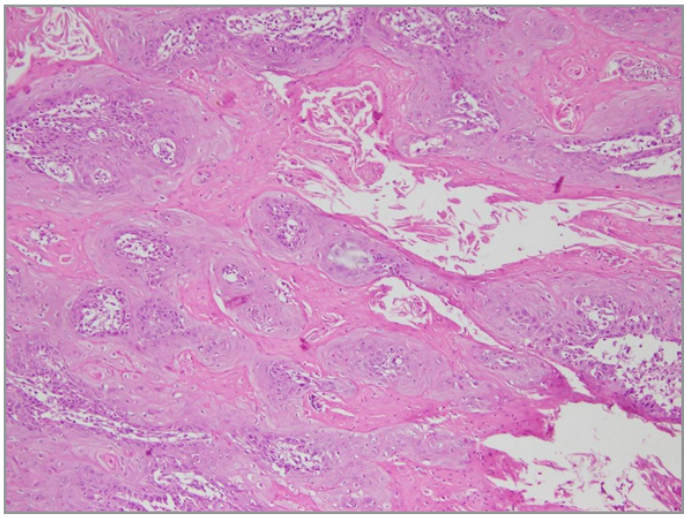

Figure 1: (Case no 46): Photomicrograph of Well Differentiated OSCC (Grade I).

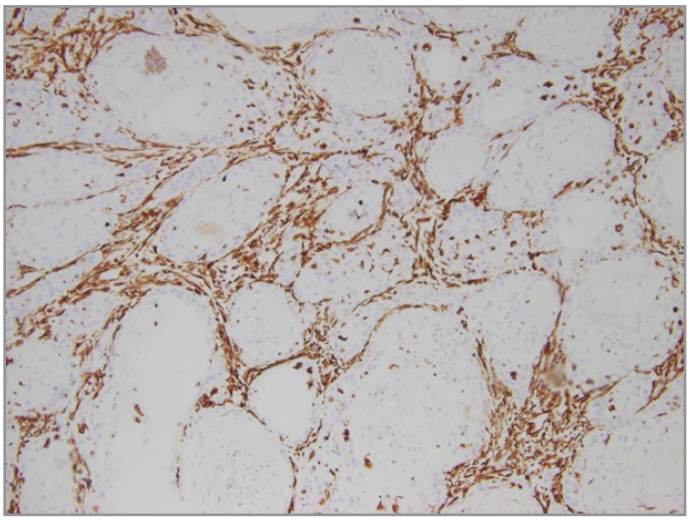

Figure 2: (Case no 17): Photomicrograph of Well differentiated OSCC with low cytoplasmic expression of vimentin

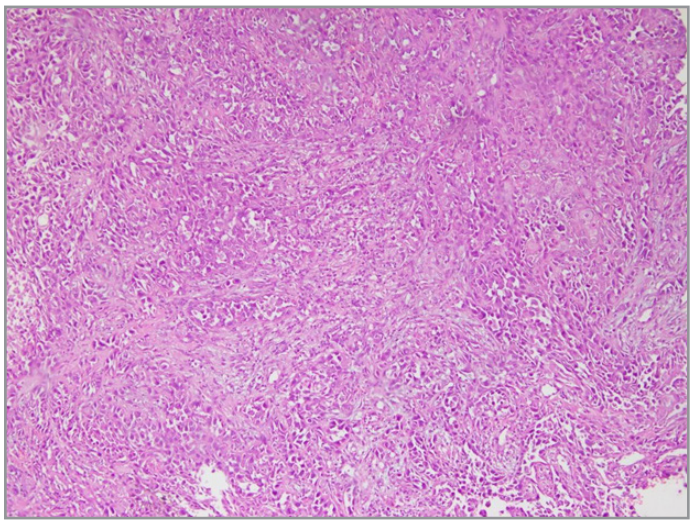

Figure 3: (Case no 49): Photomicrograph of Moderately Differentiated OSCC (Grade II).

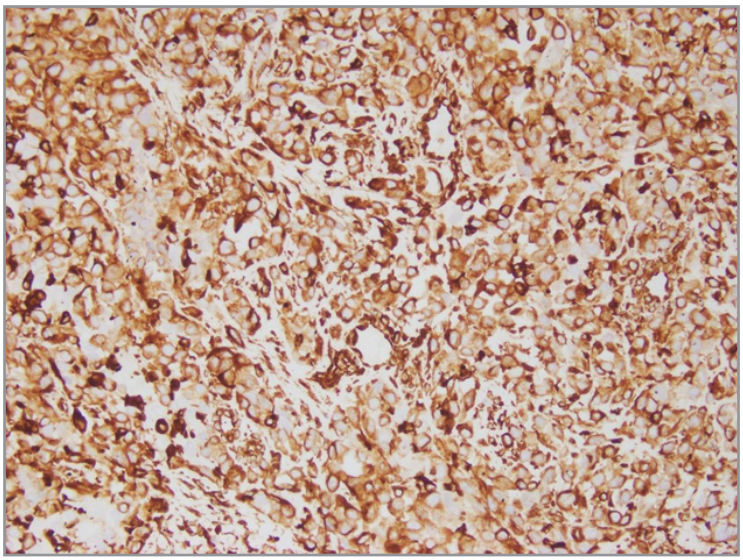

Figure 4: (Case no 49): Photomicrograph of Moderately Differentiated OSCC With High Cytoplasmic Expression of Vimentin

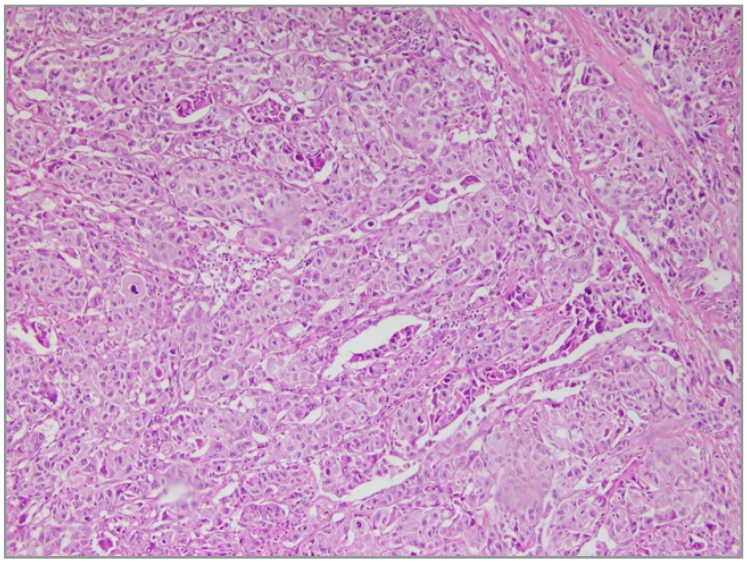

Figure 5: (Case no 51): Photomicrograph of Poorly Differentiated OSCC (Grade III).

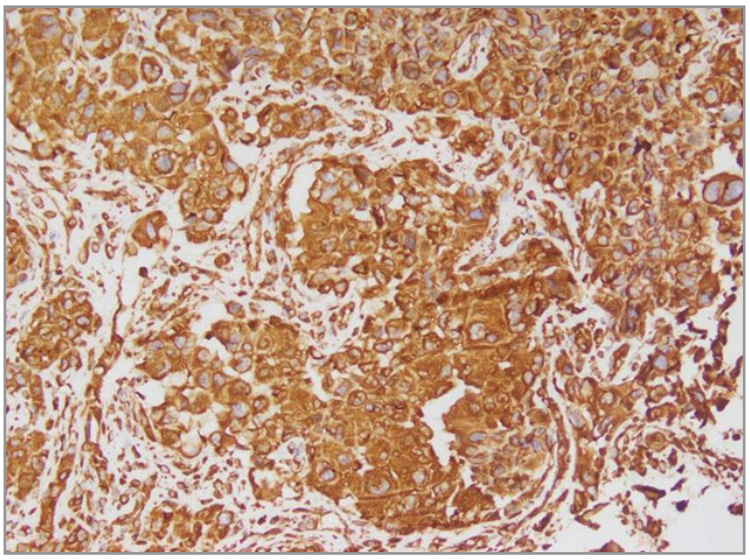

Figure 6: (Case no 51): Photomicrograph of Poorly Differentiated OSCC with High Cytoplasmic Expression of Vimentin

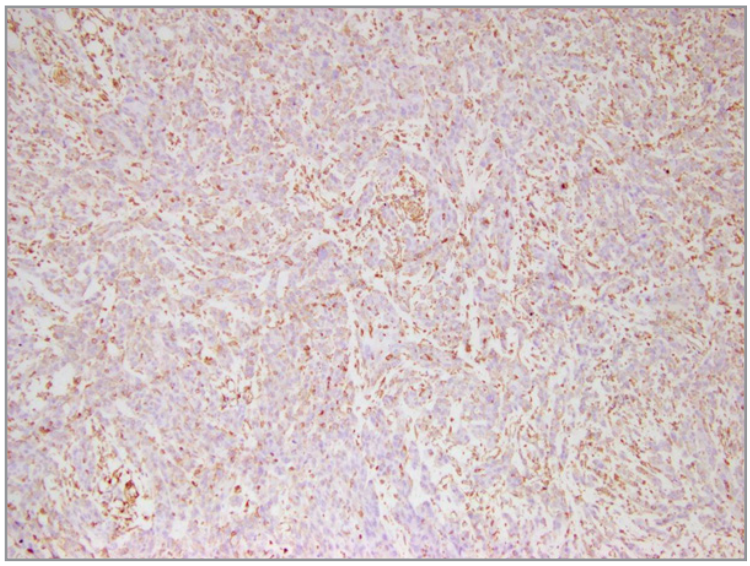

Figure 7: (Case no 3): Photomicrograph of Poorly Differentiated OSCC with Low Cytoplasmic Expression of Vimentin

Discussion

In this study, each of the six parameters of Anneroth were analyzed in excised tumor samples and WHO grade was analyzed in small biopsy samples; in association with vimentin expression to appropriately assess the significance of each of these factors. Crissman noted that a single component might have a more clinical value over the combined score of multiple histologic parameters. The chance of interobserver variability is more in WHO grading system whereas the parameters of Anneroth grading system are more reproducible [8-10].

According to the immunostaining results of the present study, more than half of the OSCC cases (53%) were categorized as having high vimentin expression score and 47% cases showed low vimentin expression scores. No case was detected as negative staining. Similar to these findings, Puneeta and her team showed positive vimentin immunoreactivity in all examined OSCC cases with high expression in 68.3% of the cases [11].

We found a significant association between vimentin expression and WHO (1971) histological grading. Some other studies established a significant association of increased expression of vimentin with WHO/Broder grading [12, 13].

Number of mitoses and lymphoplasmacytic responses were found to no significant association with vimentin expression. Crissman and his team evaluated multiple histological parameters in 77 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 and his team 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. But they stated that local recurrence or metastasis was significantly correlated with Broder's grade and degree of keratinization.14 According to this study, the association of Broder's grade, nuclear pleomorphism and degree of keratinization, with high vimentin 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 vimentin. This is one of the most important prognostic parameters of Anneroth grading system. According to Crissman, pattern of invasion was the single most important histologic variable in predicting patient survival. Odell also demonstrated pattern of invasion as a significant prognostic parameter in their study. The current study demonstrated that the frequency of high vimentin expression in association to pattern of invasion were 50%, 37%, 75% and 86% in score 1, score 2, score 3 and score 4 cases respectively. The association of the pattern of invasion with high vimentin expression was statistically significant (p value<0.05). This finding is consistent with this study by Gregory and his team [14,15].

In the present study, stage of invasion was examined in only 25 cases, excluding oral mucosal biopsy specimens and found a significant correlation between the stage of invasion and increased vimentin expression. Many studies used a cut-off value for tumor thickness and observed a significant association between increasing tumor thickness and worse patient outcomes [16].

After summation of the scores for each of the six histological parameters, total Anneroth grading was also done in 25 cases which fulfilled the criteria for all of these parameters to be examined. A significant association between increasing Anneroth grade and high Vimentin expression was found in this study.

Conclusion

Several researchers have found that Anneroth's described morphologic parameters have a better prognostic value than the conventional Broders' (1925)/ WHO grading system (1971) in predicting nodal metastasis, local recurrence, and survival. If immunohistochemical markers like vimentin is used in addition to conventional grading in the case of evaluation of OSCC cases, could be more beneficial for predicting patient outcomes as well as planning further management.

Human Ethics Declarations

This research work has been approved by Institutional Ethics Committee of Shahid Suhrawardy Medical college and Hospital, Dhaka.

Consent to Participate Declaration

Informed written consents were taken from all the patients or patient's relatives before collecting their samples for histopathological and immunohistochemical studies.

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Conflicts of Interest

None.

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