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Original Article

Immunohistochemical Expression of E-Cadherin in Normal Oral Mucosa, Precancerous Lesions and Oral Squamous Cell Carcinoma

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ABSTRACT

Oral cancer is a remarkable component of global cancer burden with significant mortality and morbidity. The present study is designed to assess the Immunohistochemical expression of E-cadherin in tissue samples of diagnosed cases of oral squamous cell carcinoma and oral premalignant lesions and normal oral mucosa.

Objective: This study aims to assess the immunohistochemical expression of E-cadherin in tissue samples of diagnosed cases of oral squamous cell carcinoma, OPMLs, and normal oral mucosa

Materials and Methods: Total of 60 cases were selected for this Descriptive cross-sectional study which included histologically diagnosed cases of OSCC (n=23), OPMDs (n=23), and Healthy individuals (n=14) as control. Immunohistochemical staining was performed for all cases. Results were analyzed using SPSS version 20.

Results: Increased expression of E – cadherin expression was found in samples of normal oral mucosa followed by samples of premalignant and malignant cases. E-cadherin expression was reduced in cases of OPMLs and OSCC as compared to normal oral mucosa. Statistically significant relation was observed while comparing the grade of staining intensity and E-cadherin expression among OSCC cases (p=0.001) and OPML cases (p= 0.01).

Conclusion: We concluded that loss of E - cadherin can be used as a tumour marker that could determine the susceptibility of normal and potentially malignant tissues to transform into oral cancers.

Key words: E-cadherin, immunohistochemistry, oral squamous cell carcinoma, oral premalignant lesions

Introduction

Globally, Oral cancer is one of the common malignant tumors.[1] In the world cancer statistics it ranks among top 15 commonest malignancies.[2] Oral cancers account for approximately 6% of the cancer incidence worldwide, with an estimated 53,260 new cases and 10,750 deaths in 2020. [3]. In Pakiatan cancer statistics it is the second most prevalent malignant tumours with marked mortality and morbidity rates.[4] Squamous cell carcinoma is the commonest histopathological variant of carcinoma of lip and oral cavity accounting for > 90% of all oral cancers.[5, 6] Mostly, invasive OSCC lesions are preceded by preinvasive stage that lasts for years. [7, 8] Progression of these preinavasive lesions to invasive carcinoma is multistage process involving the normal mucosa at one end and high grade dysplasia or CIS at the other end. [9, 10] During this period of progression, subsequently, cohesion is lost which is a result of deficiency at the molecular level. E-cadherin is a most important tumor suppressor glycoprotein that helps in cell-cell adhesion in epitheial cells. [11]

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E-cadherin is very essential to maintain the cohesion and integrity of tissues, polarity of cells, cellular growth, cellular migration and differentiation. [12, 13]

Human gene *CDH1* encodes E-Cadherin which is located on chromosome 16q22**.** It is 120 kDa glycoprotein, and a calcium dependant molecule that promotes cell to cell adhesion. [14] It is now widely recognized truth that cadherins are not just a simply 'biological glues', however generate signals from the cell surface to influence a extensive variety of cell functions which includes apoptosis, growth factor receptor activation, tumor development and metastasis. [13]

Epidemiological studies have showed that immunohistochemical E-cadherin expression is higher in normal oral mucosa, followed by oral potential malignant lesions and oral SCC. By further progression in severity of dysplasia from mild to severe, the expression of E-cadherin in OED reduces. The reduced expression of E-cad may be a reliable indicator of increase in invasiveness of oral carcinomas.[15] Therefore, it is suggested that for timely diagnosis, detection and progression of tumors, E-cadherin can be a reliable indicator.[16]

The present descriptive cross-sectional study is designed to assess immunoreactivity of E-cadherin in formalin fixed paraffin embedded blocks (FFPE) among OSCC cases and oral potentially malignant lesions. This would help in exploring its biological behavior in OSCC and oral potentially malignant lesion. It would determine its predictive role in behavior of OSCC and probable malignant potential of oral potentially malignant lesions

Materials and Methods

In this study, total sixty cases consisting of 23 cases of OSCC, 23 of OPML and 14 cases of normal oral mucosa were selected from the archival records of Department of Histopathology, Pakistan institute of Medical sciences (PIMS), Department of Oral Surgery and Oral Pathology, Peshawar Dental College and Peshawar, Department of histopathology, Peshawar Medical college with their personal history forms, after approval of ethical review committee. Duration of study was 6 months from 4thFebruary to 4th July, 2020. Non-probability convenient sampling technique was used. The cases were assesses by two histopathological grading system (WHO and Invasive tumor front grading system) for OSCC and two histopathological grading systems (WHO and Binary system) for Oral potentially malignant lesions.

Double headed light microscope at ×10 and ×40 was used for evaluation of E-cadherin positive. Sections were assessed for E-Cadherin expression in terms of intensity and site by using keys designed by Simionescu et al., [17] Key for intensity (0 or (-) absence of staining, 1 or (+) slight staining, 2 or (++) moderate staining, 3 or (+++) marked staining. Key for area of expression was graded as percentage of positive cells: Grade 1= 1-25%, Grade 2= 26-50%, Grade 3= 51-75% , Grade 4 = > 75% . Analysis of results was done by using SPSS 20. The study variables were compared by chi-square and fischer test with p-value.

Results

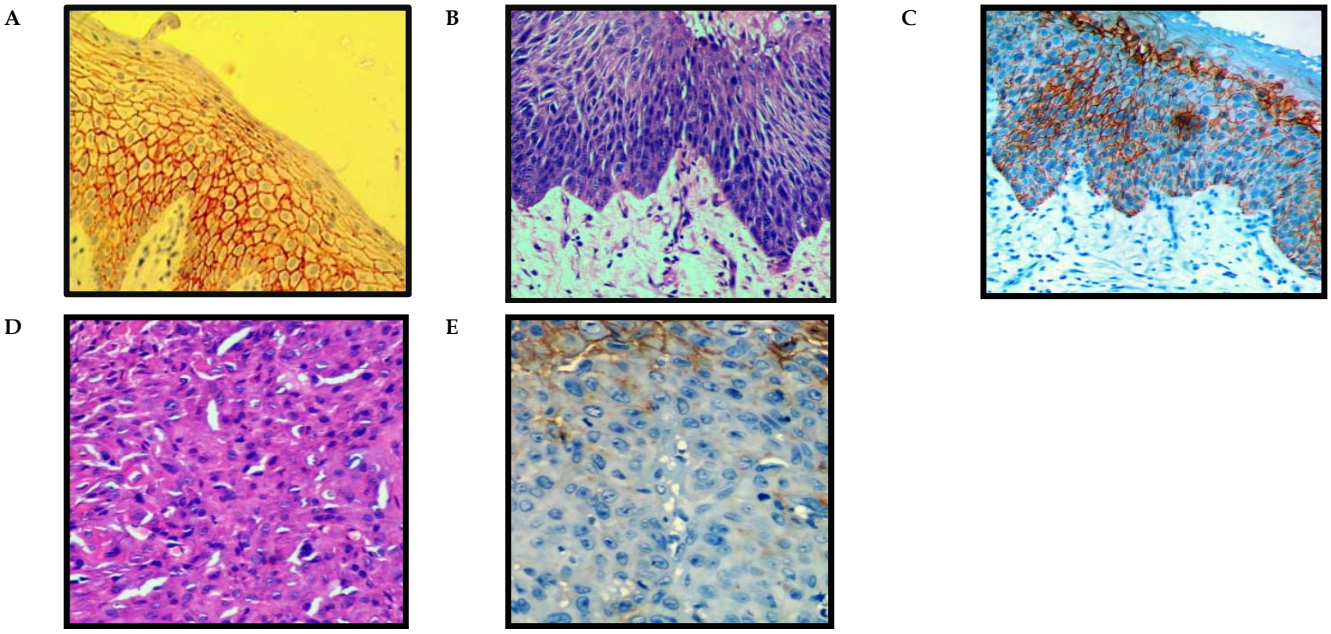
The mean age of cases of OSCC, OPML and Healthy individual was 56.39 (±12.74), 60.7(±11.092) and 49.07 (±13.135) respectively (Table 1) with statistically significant differences was observed for mean age (p=<0.05) (Table 1). Most of the cases of OSCC (n=18) and OPMLs (n=12) presented in old age i.e >50 years. Statistically significant relation was observed between the old age group among the cases and healthy individual. The observed male to female ratio for cases of OSCC, OPMLs and healthy individual was 1:1.9, 1:1.3 and 2:1.5 respectively (Table 1). Statistically insignificant relation was observed among the study participants for gender. The commonest site for development of OSCC and OPML was buccalmucosa, vestibule of mouth and retromolar area (n=9: 39.1%) and (n= 13; 56.5%) (Table 1). According to WHO grading system the most commonly occurring histological type was WDSCC (60.9%) followed by MDSCC (30.4%) and PDSCC (8.7%). marked (30.4%) (Table 2). While using ITF grading system the most commonly occurring grade of malignancy was (G2 n= 13 and G3 n= 7) (Table 2). Continuous rim and many large patches of lymphoplasmacytic infiltrate (LPI) was noted in the lesions of OSCC. The most commonly occurring grade of Lymphoplasmacytic infiltrate (LPI) were slight (39.1%), followed by moderate (30.4%) and was Moderate dysplasia (n=6; 26%) and Squamous cell hyperplasia (n=11; 47.8 %) (Table 2). The sub epithelial inflammatory infiltrate mostly comprise of acute on chronic inflammation (60.9 %) (Table 2). Among the cases of OPML all the lesions diagnosed as squamous hyperplasia (n=11; 47.8%), mild dysplasia (n=3; 13%), moderate dysplasia (n=6; 26.1%), severe dysplasia (n=2; 8.7%) expressed E-cadherin expression (Table 3). In group B, n=3; 25% of OPMLs, E-cadherin immunoreactivity showed low risk lesion and n=9; 75% of OPMLs, E-cadherin immunoreactivity showed high risk lesions (Table 3).

Among Healthy individual, E-cadherin is expressed in all the cases of normal oral mucosa (100%) (Table 4). Strong staining of E-cadherin is seen in the basal cell layer and parabasal epithelial cells layers, less staining is observed in epithelial cells of superficial layers which is indicative of normal desquamation (figure 1, 2). Statistically insignificant relation (>0.05) was observed for E-Cadherin expression among the cases of OSCC, OPML and normal oral oral mucosa (Table 7).

Among cases of OPML, all 23 cases (100%) showed E-cadherin expression. Among 23 subjects of OPML group, 8 (34.7%) showed 1+ (slight staining), 13 (56.5%) showed 2++ (moderate staining) and remaining 2 (8.6%) showed 3+++ (marked staining) (Figure 1). Statistically significant relation p=0.01, was observed while comparing the grades of staining intensity and E-cadherin expression in group B (Table 5).

Among cases of OSCC, only 1 case (4.3%) did not expressed E-cadherin expression while remaining 22 cases (95.7%) expressed E-cadherin staining. In 23 subjects of OSCC group, 11subjects (47%) showed 1+ (slight staining), 7 subjects (30.4%) showed 2++

(moderate staining) and remaining 4 subjects (17.4%) showed 3+++ (marked staining) (fig 1). Statistically significant relation (p=0.001) was observed, while comparing the grades of staining intensity and E-cadherin expression in group A **(**Table 6).



a)- E-cadherin, Immunohistochemical expression In normal oral mucosa (IHC, 40X), Showing very dark brown membranous staining **(b)-**Photomicrograph showing severe dysplasia. Cells in more than 2/3rd of the epithelium show loss of polarity, marked anisonucleosis, nuclear pleomorphism and hyperchromasia. The cells in superficial layer show maturation with keratinization (H&E, 40X). **(c)-** Photomicrograph showing , severe dysplasia E-cadherin expression is reduced in all the layers with only focal areas and few cells in upper prickle layer showing normal staining (IHC,40X). **(d)-** Higher magnification of poorly differentiated SCC showing tumour cells in sheets (H&E, 40X). **(e)-**E-cadherin expression showing predominantly loss of expression in tumor cells (IHC, 40X)

Table 1 Description of age, gender & site

Study variables			
AGE			
The mean age of presentation for cases of OSCC was 56.39 (SD± 12.24) The mean age of presentation for cases of OPML was 60.7 (SD± 11.092) The mean age of presentation for cases of NOM was 49.07 (SD± 13.135)			
Gender			
M:F for OSCC was 1:1.7 M:F for OPML was 1:1.3			
Study variables	**Group A OSCC**	**Group B OPML**	**Group C NOM**
SITE OF LESION			
Buccal mucosa	9(39.1%)	13(56.5%)	0
Tongue	10(43.47%)	5(21.75)	0
Lip	1(4.3%)	1(4.3%)	0
GUM	2(8.7%)	2(8.75)	14(100%)
Floor of mouth	1(4.3%)	1(4.3%)	0
Palate (Hard and soft palate,uvula)	0	1(4.3%)	0

Table 2: Clinicopathological Features of OSCC

HISTOPATHOLOGICAL FEATURES of OSCC				
WHO Grading System	**Group A OSCC**	**Group B OPML**	**Group C NOM**	**p-value***
WDSCC	-	14(60.9%)	14(60.9%)	0.004
MDSCC	-	7(30.4%)	7(30.4%)	
PDSCC	1(4.3%)	1(4.3%)	2(8.7%)	
Invasive tumour front grading system				
G1	-	3(13%)	3(13%)	0.303
G2	-	13(56.5%)	13(56.5%)	
G3	1(4.3%)	6(26.1%)	7(30.4%)	
Grades of lymphoplasmacytic infiltrate				
Marked (Continous rim)	-	7(30.4%)	7(30.4%)	0.297
Moderate (Many large patches)	1(4.3%)	6(26.1%)	7(30.4%)	
Slight (A few patches)	-	9(39.1%)	9(39.1%)	
None (No infiltration found)	-	9(39.1%)	9(39.1%)	

Table 3: Clinicopathological Features OF OPMLS

OPMLs			
Study Variables	**Binary system of OED**		**p-value***
	High Risk Lesion	**Low risk lesion**	
Oral Leukoplakia	5 (41.6%)	3 (25%)	**
Oral Erythroplakia	2(16.6%)	-	
Oral Lichen planus	-	-	
Speckled leukoplakia	2(16.6%)	-	
Total	9(75%)	3(25%)	
Degree of Oral Epithelial Dysplasia	**High Risk Lesion**	**Low risk lesion**	**0.13**
Mild dysplasia	1(8.3%)	2(16.6%)	
Moderate dysplasia	5(41.6%)	1(8.3%)	
Severe dysplasia/CIS	3(25%)	-	
Total	9(75%)	3(25%)	
*Pearson's Chi square test			
**Not computed by spss			

Table -4: E-cadherin Expression in Normal Oral Mucosa

Grades of Staining Intensity	**Marker Protein Expression**						**p-value***
	Absence of staining or no staining	**Grade 1= 1-25%**	**Grade 2= 26-50%**	**Grade 3= 51-75%**	**Grade 4= >75%**	**Total**	
No stain	-	-	-	-	-	-	**0.02**
Slight staining	-	-	-	-	-	-	
Moderate staining	-	-	-	4(28.6%)	2(14.2%)	6(42.8%)	
Strong staining	-	-	-	5(35.7%)	3(21.4%)	8(50.0%)	
Total	-	-	-	9(64.2%)	5(35.7%)	14(100%)	
*Pearson's Chi-square Test							

Table-5: E-cadherin immunohistochemical expression and staining intensity among cases of OPML

Grades of Staining Intensity	Marker Protein Expression						p-value*
	Absence of staining or no staining	Grade 1= 1-25%	Grade 2= 26-50%	Grade 3= 51-75%	Grade 4= >75%	Total	
No stain	-	-	-	-	-	-	0.01
Slight staining	-	6(26%)	2(8.7%)	-	-	8(34.7%)	
Moderate staining	-	4(17.4%)	7(30.4%)	2(8.7%)	-	13(56.5%)	
Strong staining	-	1(4.3%)	-	-	1(4.3%)	2(8.6%)	
Total	-	11(47.8%)	9(39.1%)	2(8.7%)	1(4.3%)	23(100%)	
*Pearson's Chi-square Test							

Table -6: E-cadherin immunohistochemical expression and staining intensity in OSCC

Grades of Staining Intensity	Marker Expression						p-value*
	Absence of staining or no expression	Grade 1= 1-25%	Grade 2= 26-50%	Grade 3= 51-75%	Grade 4= >75%	Total	
No staining	1(4.3%)	-	-	-	-	1(4.3%)	0.001
Slight staining	-	9(39.1%)	1(4.3%)	1(4.3%)	-	11(47.8%)	
Moderate staining	-	4(17.4%)	3(13%)	-	-	7(30.4%)	
Strong staining	-	1(4.3%)	2	-	1(4.3%)	4(17.4%)	
Total	1(4.3%)	14(60.9%)	6(26.1%)	1(4.3%)	1(4.3%)	23(100%)	
*Pearson's Chi-square Test							

Table 7: Comparison of E-Cadherin Immunoreactivity

Among tissue samples of OSCC, OPML and normal oral oral mucosa					
E-cadherin Immunoreactivity	**OSCC**	**OPML**	**Normal oral mucosa**	**Total**	**p-value***
Positive	22(95.6%)	23(100%)	14(100%)	98.3%	**0.44**
Negative	1(4.3%)	-	-	1.7%	
Total	23(100%)	23(100%)	14(100%)	100%	
*Pearson's Chi-square test					
Among tissue samples of OSCC, OPML					
E-cadherin Immunoreactivity	**OSCC**		**OPML**		**p-value***
Positive	22(95%)		23(100%)		**0.312**

Discussion

The oral cavity is lined by squamous epithelium which is subjected to both mechanical and chemical stress. [13] The epithelium shows adaptation to this stress by continuous cell renewal and differentiation.[12] Numerous molecules are involved in this process such as p53, Retinoblastoma gene, Cyclin-D and E, EGFR

and c-Jun [3] . One among them is E- cadherin [4, 5] . E-cadherin helps to maintain the cohesion and integrity of tissues, polarity of cells, cellular growth, cellular migration and differentiation. [3, 14] The abnormal expression of Ecadherin is associated with the occurrence, development and metastasis of the tumour.[14]

Oral squamous cell carcinoma (OSCC) is the most common occurring oral epithelial malignancy [5]. There is always a perpetual search for a biomarker that can help in the timely diagnosis of malignancy and to predict its prognosis as well. Early diagnosis of OSCC and its premalignant forms is the key to improve the survival rate of OSCC and prevent the risk of conversion of oral potentially malignant lesions into malignancy. In line with this concept, this study has been carried out to know the expression of E-cadherin in premalignant and malignant squamous lesions and its significance in such lesions.[6]

In our study, the mean age for cases of NOM, OSCC and OPML was 49.07 (SD± 13.135), 56.39 (SD± 12.24) and 60.7 (SD± 11.092) respectively (Table 1) .The results were in concordance with other studies. [23, 24] Silva et al., [14] reported a mean age of 57 years for both premalignant and malignant lesions, while Tran et al ., [3] reported 62 years as mean age. Male to female ratio for the group of OSCC and OPML was 1:1.7 and 1:1.3 respectively, which was contrary to results shown by Awan et al., [6] and Akhtar et al.,[23] respectively (Table 1).

In the present study, commonest site for both premalignant and malignant lesions was the buccal mucosa (Table 1) which was similar to the results reported by Talukdar & Goswami [24] and Awan et al [6] . However Silva et al [14] reported tongue as the most common site for malignancy in their study. Overall, the most common site for the premalignant and malignant lesions in Pakistan population is buccal mucosa. [6]

All the cases of normal oral mucosa showed a very strong expression of E-cadherin. Strong continuous membranous staining of E-cadherin was observed in basal and parabasal layer epithelial cells and reduce staining is observed in epithelial cells of superficial layers which is indicative of normal desquamation. Similar observations were also observed by other studies [14, 22] (figure 1b, c). Statistical significant value (p=0.02) was observed in E-cadherin immunohistochemical expression and staining intensity among cases of OPML (Table 4).

Histopathologically , Squamous cell hyperplasia (47.8 %) was commonly occurring epithelial precancerous lesion followed by mild, moderate and severe dysplasia. Our study revealed results (Table 1) which is consistent with other studies Kaur et al., [26] but contrary to findings of study done by A. Hamaamin et al., [27].

Out of total 23 cases of OPMLs, n=8 (34%) sowed slight staining, n=13(56.5%) shows moderate staining, and only 2 cases showed strong attaining in terms of intensity. It was observed in the present study that there was down regulation in expression of E-cadherin as severity of dysplasia increases. Statistical significant value (p=0.01) was observed in E-cadherin immunohistochemical expression and staining intensity among cases of OPML (Table 5). This observation was consistent with observation of the previous studies [10, 14]. The reduction in expression of E-cadherin was less in the mild and moderate degree of dysplasia, as compared to severe degree of dysplasia. In cases of mild epithelial dysplasia E-cadherin expression was present in supra basal and basal similar to the normal epithelium while E-cadherin expression in cases of moderate dysplasia was present in supra basal but expression was reduced in the basal cell layer (figure 1a). This loss leads to acquisition of invasive property by the basal cell . [14, 15] Sridevi et al.,[25] reported weak expression of E-cadherin in 75% of the cases of leukoplakia with mild dysplasia, and weak and loss of expression in 75% of the cases of OSF with mild dysplasia.

Oral SCC were graded after analyzing expression of E-Cadherin on the basis of WHO and invasive tumour front grading system. This present study showed the most cases of OSCC presented in G2 (56.5%) grade of ITF grading system (Table 2), which is in accordance to other studies reported by Mehendiratta et al.,[12]. Among 23 cases of our study 14 (60.9%) were well differentiated, 7 (30.4 %) were moderately differentiated and 2 (8.7 %) were poorly differentiated. Down regulation in intensity of expression of E-Cadherin was observed as there is increase in grades of OSCC (figure 1 d,e). The staining intensity was gradually decline significantly as the disease advances in severity. 11 (47%) slight (+), 7 (30.4%) moderate (++), 4 (17.4%) marked (+++) and 1 (4.3%) negative (0) cases of E-Cadherin expression were observed out of 23 OSCC cases .14 (60%) cases show grade 1 (1-25%) immunohistochemical expression of E-cadherin, 6 (26%) showed grade 2 (26-50%), 1 (4.3%) showed grade 3 (51-75%) and only 1 (4.3%) showed grade 4 (>75%). Statistical significant value (p=0.01) was observed in E-cadherin immunohistochemical expression and staining intensity among cases of

OSCC (Table 6). These results have also been reported by various researchers. [5, 29] It has been encountered as tumour become poorly differentiated, it lost the membranous pattern and found as faint cytoplasmic staining. These observations are in accordance to various studies [1, 4, 9, 18] .

Conclusion

Present study revealed the down-regulation of the molecular marker E-cadherin in cases of OPML and OSCC in comparison to normal oral mucosa cells. Hence, it was concluded by our study that E-cadherin could be of clinical importance in OSCC and can be used for improving the diagnosis and prognosis of OSCC by probable indication of malignant potential of OPMLs by loss in its immunohistochemical expression in premalignant and malignant cases progressively.

Recommendations and Future directions:

To generalize our results, further prospective studies with a large sample size using advance molecular technique such as PCR, cell culture and gelatin zymography to read the gene

Conflict of interest: None.

References

1. Al-Jaber A, Al-Nasser L, El-Metwally A. Epidemiology of oral cancer in Arab countries. Saudi medical journal. 2016;37(3):249.
2. Ghantous Y, Abu Elnaaj I. [GLOBAL INCIDENCE AND RISK FACTORS OF ORAL CANCER]. Harefuah. 2017;156(10):645-9.
3. Tran CM, Kuroshima T, Oikawa Y, Michi Y, Kayamori K, Harada H. Clinicopathological and immunohistochemical characteristics of pigmented oral squamous cell carcinoma. Oncology Letters. 2021;21(4):1-9.
4. Karaca I, Ozturk D. Oral cancer: Etiology and risk factors. Journal of cancer research and therapeutics. 2019;15(3):739-.
5. Awan AM, Naz I, Mahmood MK, Uddin H. Comparison of expression of E-cadherin in oral Pseudoepitheliomatous hyperplasia and Oral squamous cell carcinoma. 2019.
6. Givony S. Oral squamous cell carcinoma (OSCC) an overview. Medicinos mokslai Medical sciences Kėdainiai: VšĮ" Lietuvos sveikatos mokslinių tyrimų centras", 2020, vol 8, no 13, Mar 23. 2020.
7. Yousaf A, Mahmood S, Faraz R, Quader Q. Annual cancer registry report-2018, of the Shaukat Khanum Memorial Cancer Hospital & Research Center, Pakistan. 2019.
8. Richards D. Prevalence of oral potentially malignant disorders. Evidence-based dentistry. 2018;19(4):120-1.
9. GuPta K, MauRYa MK, MEhROtRa R, GOEL M, KuMaR M, JaISwaL R, et al. Utility of E-Cadherin as a Prognostic Marker in Oral and Cervical Squamous Cell Neoplastic Lesions. Journal of Clinical & Diagnostic Research. 2018;12(1).
10. Iocca O, Sollecito TP, Alawi F, Weinstein GS, Newman JG, De Virgilio A, et al. Potentially malignant disorders of the oral cavity and oral dysplasia: A systematic review and meta-analysis of malignant transformation rate by subtype. Head & neck. 2020;42(3):539-55.
11. Petrova YI, Schecterson L, Gumbiner BM. Roles for E-cadherin cell surface regulation in cancer. Molecular biology of the cell. 2016;27(21):3233-44.
12. Mehendiratta M, Solomon MC, Boaz K, Guddattu V, Mohindra A. Clinico-pathological correlation of E-cadherin expression at the invasive tumor front of Indian oral squamous cell carcinomas: An immunohistochemical study. Journal of oral and maxillofacial pathology: JOMFP. 2014;18(2):217.
13. Shapiro L. Structure and Function of Cadherin Extracellular Regions. The Cadherin Superfamily: Springer; 2016. p. 71-91.
14. Silva ADd, Maraschin BJ, Laureano NK, Daroit N, Brochier F, Bündrich L, et al. Expression of E-cadherin and involucrin in leukoplakia and oral cancer: an immunocytochemical and immunohistochemical study. Brazilian oral research. 2017;31.
15. Du Y, Li H. Expression of E-cadherin in oral lichen planus. Experimental and therapeutic medicine. 2015;10(4):1544-8.
16. Fernandez A, Marshall M, Santibanez N, Martínez C, Fernandez J, Martínez R. Comparing the immuno-expression of endothelial markers in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma: Relationship between E-cadherin, vimentin, CD31, CD117 and epithelial-mesenchymal transition. MOJ Anat Physiol. 2017;4:00135.
17. Simionescu C, Mărgăritescu C, Surpăţeanu M, Mogoantă L, Zăvoi R, Ciurea R, et al. The study of E-cadherine and CD44 immunoexpression in oral squamous cell carcinoma. Rom J Morphol Embryol. 2008;49(2):189-93.
18. Langhe RP, Gudzenko T, Bachmann M, Becker SF, Gonnermann C, Winter C, et al. Cadherin-11 localizes to focal adhesions and promotes cell–substrate adhesion. Nature communications. 2016;7(1):1-10.
19. Vajaria BN, Patel KR, Begum R, Shah FD, Patel JB, Joshi GM, et al. Correlation between Loss of E-cadherin, Matrix-metalloproteinases and c-Jun expression in Oral Carcinogenesis. American Journal of Oral Medicine. 2017;3(1):1-24.
20. Gloushankova NA, Rubtsova SN, Zhitnyak IY. Cadherin-mediated cell-cell interactions in normal and cancer cells. Tissue barriers. 2017;5(3):e1356900.

21. Smitha A, Rao K, Umadevi H, Smitha T, Sheethal H, Vidya M. Immunohistochemical study of α-smooth muscle actin expression in oral leukoplakia and oral squamous cell carcinoma. Journal of oral and maxillofacial pathology: JOMFP. 2019;23(1):59.
22. Gupta A, Sharma S, Batra M, Abidullah M, Bhuvinder S, Katragadda P. Role of E-cadherin in Progression of Oral Squamous Cell Carcinoma: A Retrospective Immunohistochemical Study. The journal of contemporary dental practice. 2018;19(9):1105-10.
23. Akhtar K, Ara A, Siddiqui SA, Sherwani RK. Transition of Immunohistochemical Expression of E-Cadherin and Vimentin from Premalignant to Malignant Lesions of Oral Cavity and Oropharynx. Oman medical journal. 2016;31(3):165-9.
24. Leena T, Anirban G. An immunohistochemical study of e-cadherin expression in primary oral squamous cell carcinoma and metastatic lymph node in a tertiary care centre. Tropical Journal of Pathology and Microbiology. 2019;5(11).
25. Sridevi U, Jain A, Nagalaxmi V, Kumar UV, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. Eur J Dent. 2015;9(3):364-72.
26. Kaur J, Sawhney M, DattaGupta S, Shukla NK, Srivastava A, Walfish PG, et al. Clinical significance of altered expression of β-catenin and E-cadherin in oral dysplasia and cancer: potential link with ALCAM expression. PLoS One. 2013;8(6):e67361.
27. Hamaamin K, Hussein F, Mohammad D. Immunohistochemical Expression of PTCH1 and Laminin in Oral Hyperplastic, Premalignant, Squamous Cell Carcinoma and Recurrence Lesions Samples. Sulaimani dental journal. 2019;6.
28. Siriwardena SB, Tsunematsu T, Qi G, Ishimaru N, Kudo Y. Invasion-related factors as potential diagnostic and therapeutic targets in oral squamous cell carcinoma—a review. International journal of molecular sciences. 2018;19(5):1462.
29. Invasiveness Avpo. in histological grades of oral squamous cell carcinoma. Pakistan Oral & Dental Journal. 2013;33(2).
30. Sridevi U, Jain A, Velpula Nagalaxmi UVK, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. Eur J Dent. 2015;9(3):364.
31. Kafil Akhtar AA, Siddiqui SA, Sherwani RK. Transition of immunohistochemical expression of e-cadherin and vimentin from premalignant to malignant lesions of oral cavity and oropharynx. Oman medical journal. 2016;31(3):165.

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A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion