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Immunohistochemical Expression Of E-Cadherin In Various Grades Of Oral Epithelial Dysplasia And Oral Squamous Cell Carcinoma

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ABSTRACT

Background: As oral cancer is considered as a 6th most common malignant neoplasm worldwide this represents about 3% of all head and neck of cancers. Oral pre cancerous lesions are more prevalent nowadays and their malignant transformation is a multi-step processes that involves a genomic alterations. Hereby, E-cadherin is an important tumor suppressor gene and cell adhesion molecules it has been reported that decreased expression of E-cadherin /catenin complex has been reported to one of the main factor in cancer progression.

Aim: To evaluate and compare the immunohistochemical expression of E-cadherin/catenin between various grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC)

Methodology: Total of 35 archivaly retrieved paraffin embedded tissues blocks were studied for E- cadherin expression using IHC. Group A: Control Group –Normal Mucosa (N = 5), Group B: OED (N=15), Mild Dysplasia-5, Moderate Dysplasia - 5, Severe Dysplasia -5, Group C: OSCC (N=15), OSCC (Well differentiated) -5, OSCC (Moderately differentiated) -5, OSCC (Poorly differentiated) -5

Result: strong positivity (40%) in 2 cases of mild dysplasia and only (20 %) in 1 case of severe dysplasia, moderate positivity (60%) in 3 cases of mild and 3 cases of moderate dysplasia and mild positivity (40%) in 2 cases of moderate dysplasia and (80%) in 4 cases of severe dysplasia. The decreased expression of E-cadherin was noted from mild to severe degree of dysplasia and in increasing grades of OSCC with a significant p value of 0.004.

Conclusion: decreased E-cad expression cause alterations in epithelial characteristics of the cells which finally lead to higher invasiveness. This facilitates early diagnosis and increases patient survival rate.

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I. INTRODUCTION

Oral cancer are 6th most common malignant neoplasm worldwide which represents 3% of all head and neck of cancer (1, 2) Oral squamous cell carcinoma (OSCC) comprises approximately 90% of all oral malignancies. (3, 4) Pindborg JJ et al, in 1997 first defined OSCC based on its characterization in formation of keratin pearl. (5) The cause of oral cancer is multifactorial, which includes both intrinsic and extrinsic factors. 80% of OSCC cases have been reported to be associated with precancerous lesions. (6, 7) The world wide prevalence of oral potentially malignant disorders (OPMD) such as Erythroplakia, leukoplakia, erosive lichen planus, lupus erythematosus and Reverse smoker's palate have been reported to be around 1-5%. Malignant transformation of OPMD is multi-step processes were it involves a genomic alterations. (7, 8) H &

E stained sections assesses the architectural and cytological changes which can be evaluated microscopically and histopathological diagnosis of OPMD includes hyperplasia, hyperkeratosis to oral epithelial dysplasia (OED). The exact mechanisms of a dysplastic lesions undergoing malignant transformation and progressing into cancer are poorly understood. The balancing mechanism between cellular adhesion and motility is responsible for tumor initiation and progression, Cell-cell adhesion plays a vital role which is promoted by E-cadherin/β-catenin complexes. (11, 12, 13)

E-cadherin is a important tumor suppressor gene and cell adhesion molecules, which are encoded by CDH1 gene located on the chromosome 16q21, and which reported to have a major role in establishing cell polarity and to maintain the normal tissue architecture. Decreased or loss of expression of E- cadherin /catenin complex has been reported to one of the factor in cancer progression. (8, 13, 14) Currently there is no strong affirmation for the use of tumor markers to study the progression of oral epithelial dysplasia to OSCC.

The current study was attempted to evaluate and compare the immunohistochemical expression of E-cadherin/catenin between various grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC), which can be a reliable marker in identifying the invasiveness of oral carcinomas and also it has been reported that poor prognosis in association with oral cancer.

II. MATERIALS AND METHODOLOGY:

Total of 35 archivaly retrieved paraffin embedded tissues blocks were studied for E- cadherin expression using IHC. The tissue samples were grouped into: Group A: Control Group –Normal Mucosa (N = 5), Group B: OED (N=15), Mild Dysplasia-5, Moderate Dysplasia - 5, Severe Dysplasia -5, Group C: OSCC (N=15), OSCC (Well differentiated) -5, OSCC (Moderately differentiated) -5, OSCC (Poorly differentiated) -5. Immunoreactivity of E-cadherin expression was analyzed based on the intensity and area of staining.

IHC METHODOLOGY

The archived tissues were sectioned to 3µm thickness are mounted on positively charged slides and kept for overnight incubation at 37℃. Routine IHC staining was done using streptavidin Avidin -Biotin complex (S-ABC) labeling technique and visualized with diaminobenzidine tetrahydrochloride (DAB). Dewaxed sections were washed in alcohol; using pressure cooker technique by Tris -Ethylene di-amine tetra acetic acid (Tris – EDTA) at pH 7 for 15- 20 minutes (at 200̊c up to 2 whistles in pressure cooker) antigen retrieval was done.

Sections were finally washed in Tris buffered saline, endogenous peroxidase blocking using Poly excel H2O2 was done and kept for 5 minutes. Again it is washed with immuno wash buffer and incubated in a humidifying chamber with primary antibody for 45 minutes. Slides were again washed in TRIS wash buffer for 5 minutes by 3 changes and incubated with secondary antibody poly excel target binder reagent for 12minutes and washed with 3 changes of TRIS wash buffer for 5 minutes. For visualization, sections were incubated with Poly excel HRP for 12 minutes and washed subsequently. Then DAB solution was added and kept for 2-5 minutes, finally the sections were washed in distilled water and counterstained with Harris hematoxylin for 30 seconds, washed in running tap water for 5 minutes dried and mounted with DPX.

STATISTICAL ANALYSIS

All the positive and negative values were tabulated and entered in Excel sheet, calculations were performed using SPSS version 21.0 and the final values were analysed by Kruskall Wallis test and p value <0.05 was considered as staistically significant.

RESULTS:

INTENSITY OF STAINING :

Total 15 cases of OED: 5 were mild, 5 were moderate and 5 were severe dysplasia. E-cad expression, showed strong positivity (40%) in 2 cases of mild dysplasia and only (20 %) in 1 case of severe dysplasia, moderate positivity (60%) in 3 cases of mild and 3 cases of moderate dysplasia and mild positivity (40%) in 2 cases of moderate dysplasia and (80%) in 4 cases of severe dysplasia .

Total 15 cases of OSCC: 5 were well differentiated, 5 were moderately differentiated and 5 were poorly differntiated. E-cad expression, showed strong positivity (80 %) in 4 cases of well differentiated OSCC and (60%) in 3 cases of moderately differentiated OSCC, mild positivity (40%) in 2 cases of moderately differentiated OSCC and (80%) in 4 cases of poorly differentiated OSCC negative expression was noted in only (20%) 1 case of poorly differentiated OSCC. (Fig 1-6)

In normal mucosa (80%) in 4 cases showed intense staining and only (20%) in 1 case showed moderate staining.

The decreased expression of E-cadherin was noted from mild to severe degree of dysplasia and in increasing grades of OSCC with a significant p value of 0.004. (Table 1, 2, 3, 4)

Figures:

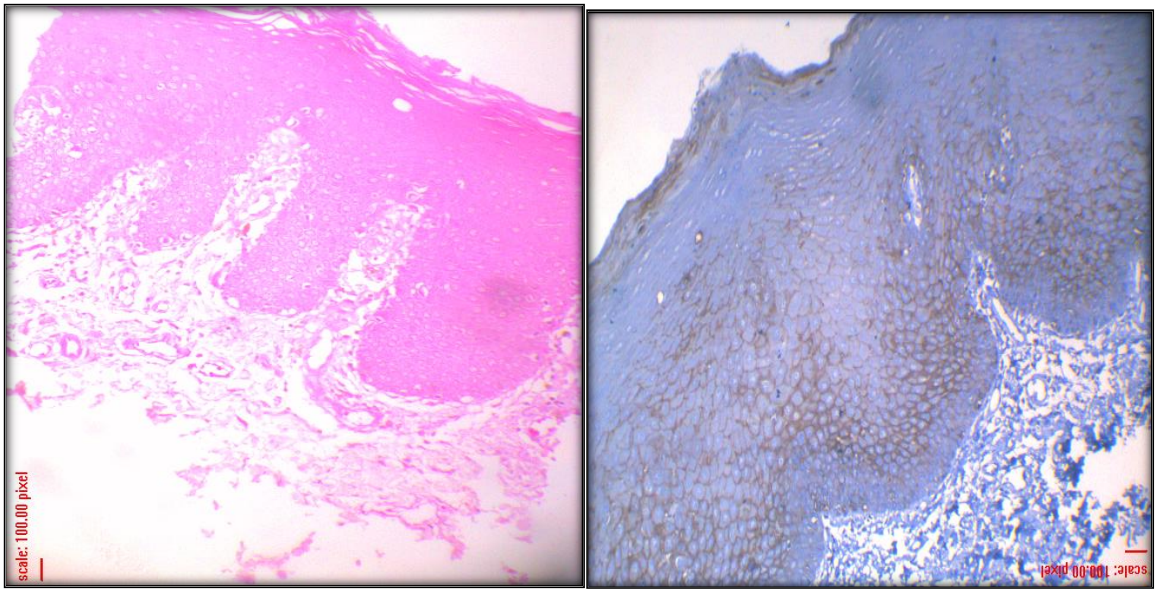


Fig 1: (10x) Mild Dysplasia Section Reveals Intense Staining, Strong Expression of E-Cadherin

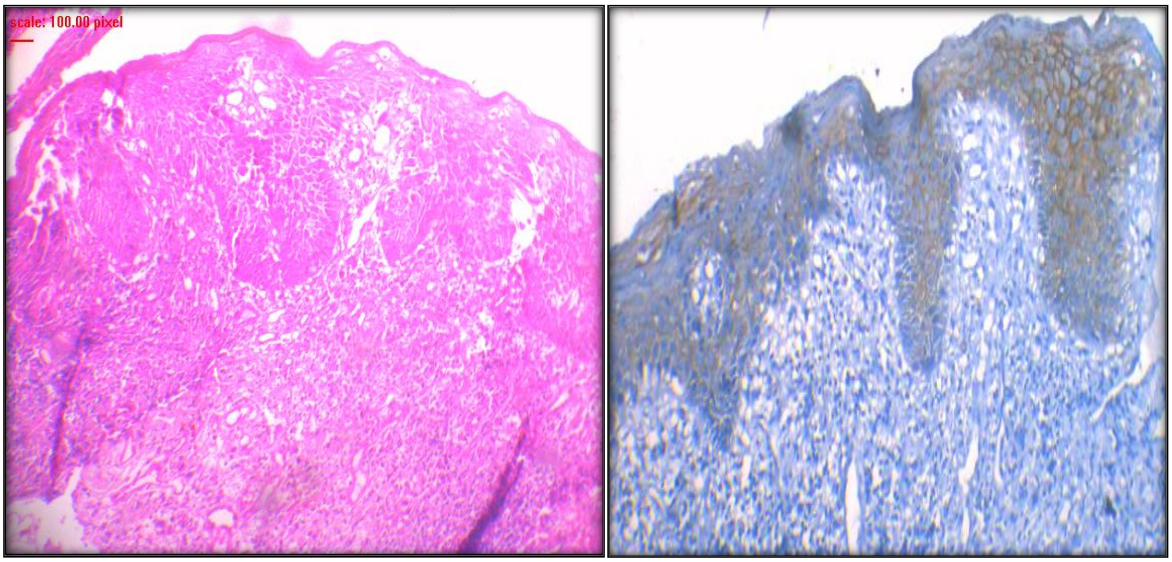


Fig 2: (10X) Moderate Dysplasia Section Reveals Moderate Expression of E-Cadherin

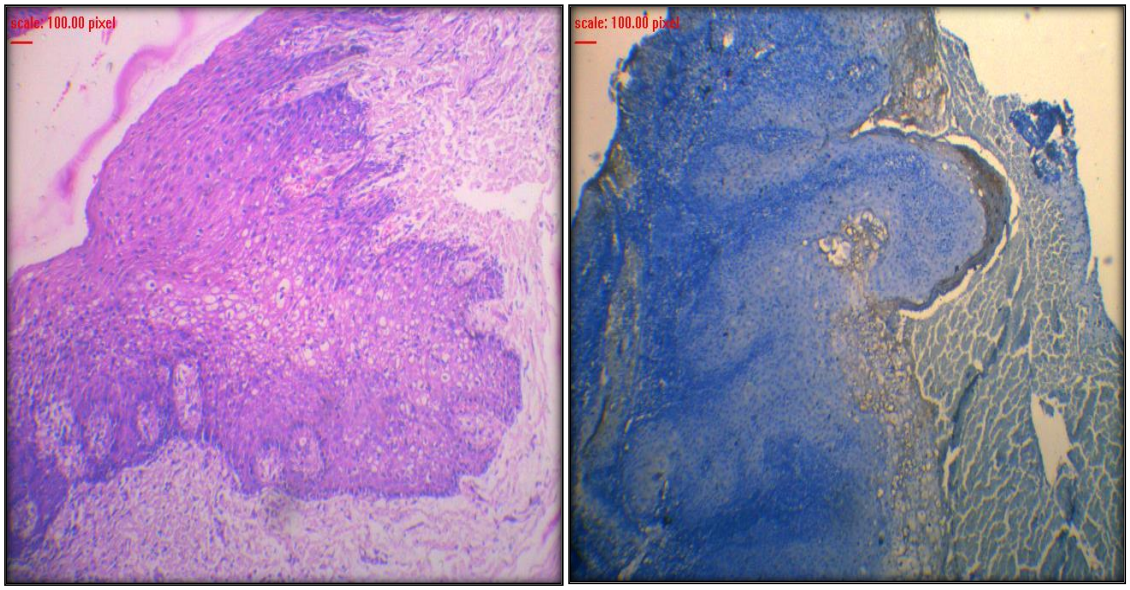


Fig 3: (10X) Severe Dysplasia Section Reveals No Expression of E-Cadherin

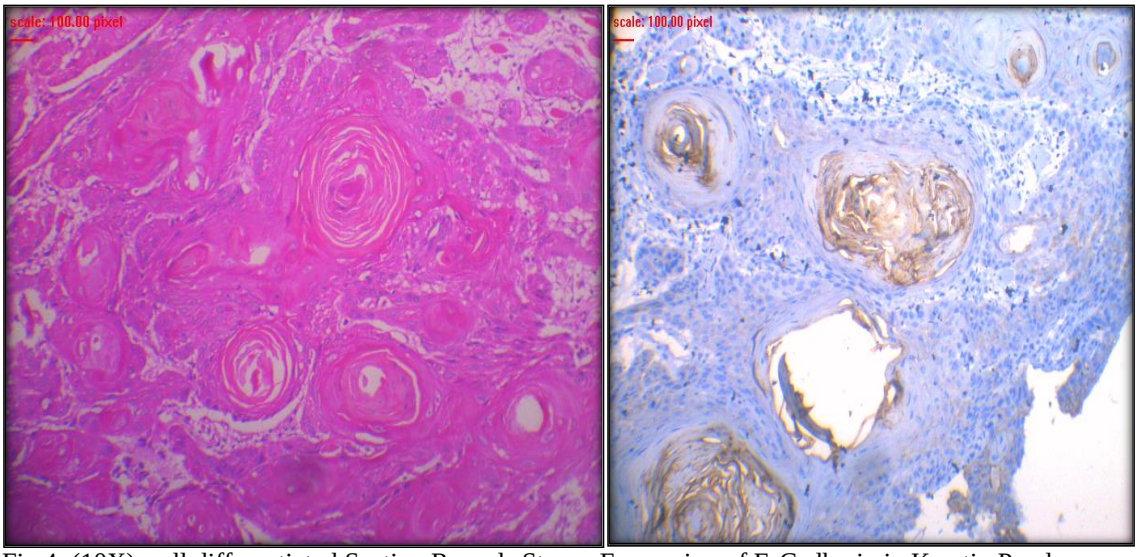


Fig 4: (10X) well differentiated Section Reveals Strong Expression of E-Cadherin in Keratin Pearls

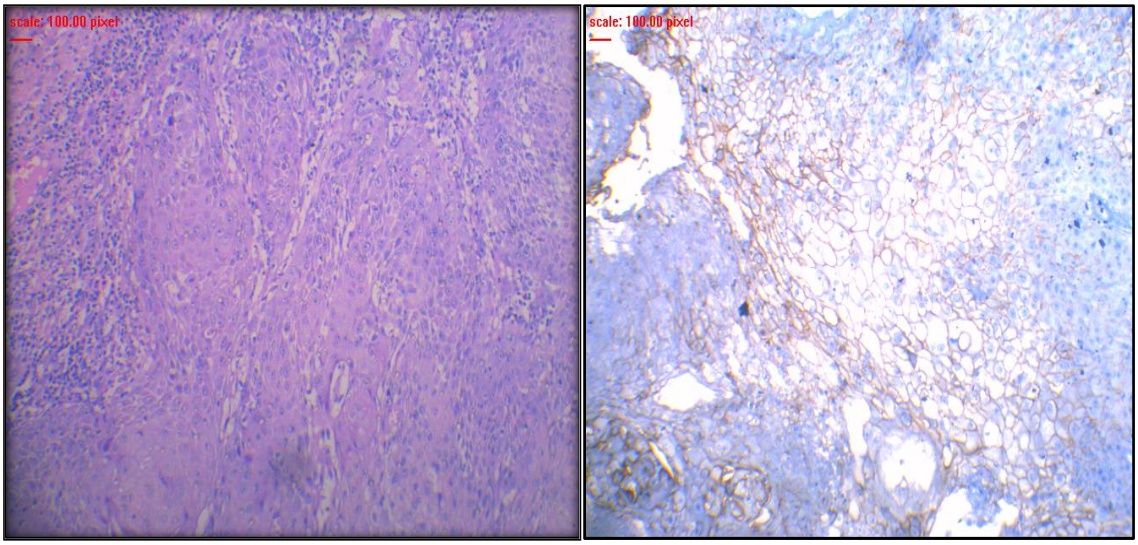


Fig 5: (10X) moderately differentiated Section Reveals Mild Expression of E-Cadherin

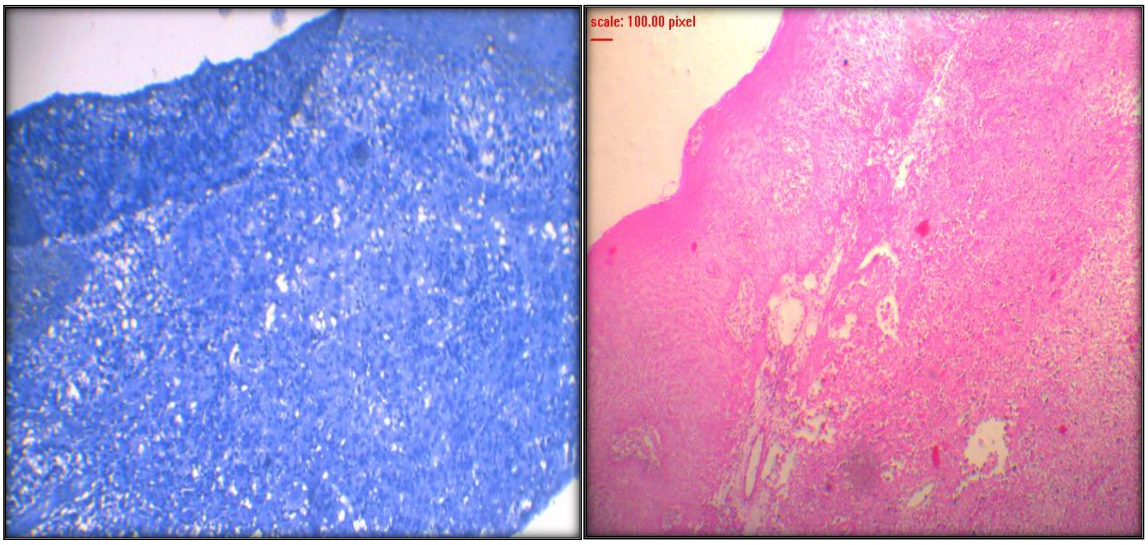


Fig 6: (10X) poorly differentiated Section Reveals No Expression of E-Cadherin

RESULTS:
Tables:

EVALUATION OF IMMUNOREACTIVITY FOR E-CADHERIN: (TABLE 1)

INTENSITY OF STAINING	AREA OF STAINING
0 -No staining	1<25 %
+1 -Mild staining	2-25% to 50 %
+2 -Moderate staining	3- 50% to 75%
+4 – Intense staining	4- >75%

EXPRESSION OF E-CADHERIN IN NORMAL MUCOSA(Group- A Control group) (TABLE 2)

INTENSITY OF STAINING (SCORE)	NORMAL MUCOSA Total (n=5)	AREA OF STAINING (SCORE)	NORMAL MUCOSA Total (n=5)
0	0	1	0
+1	0	2	0
+2	1	3	2
+3	4	4	3

EXPRESSION OF E-CADHERIN IN ORAL EPITHELIAL DYSPLASIA : (Group B) (TABLE 3)

INTENSITY OF STAINING	MILD (N =5)	MODERATE (N=5)	SEVERE (N=5)
0	0	0	0
+1	0	2	4
+2	3	3	0
+3	2	0	1

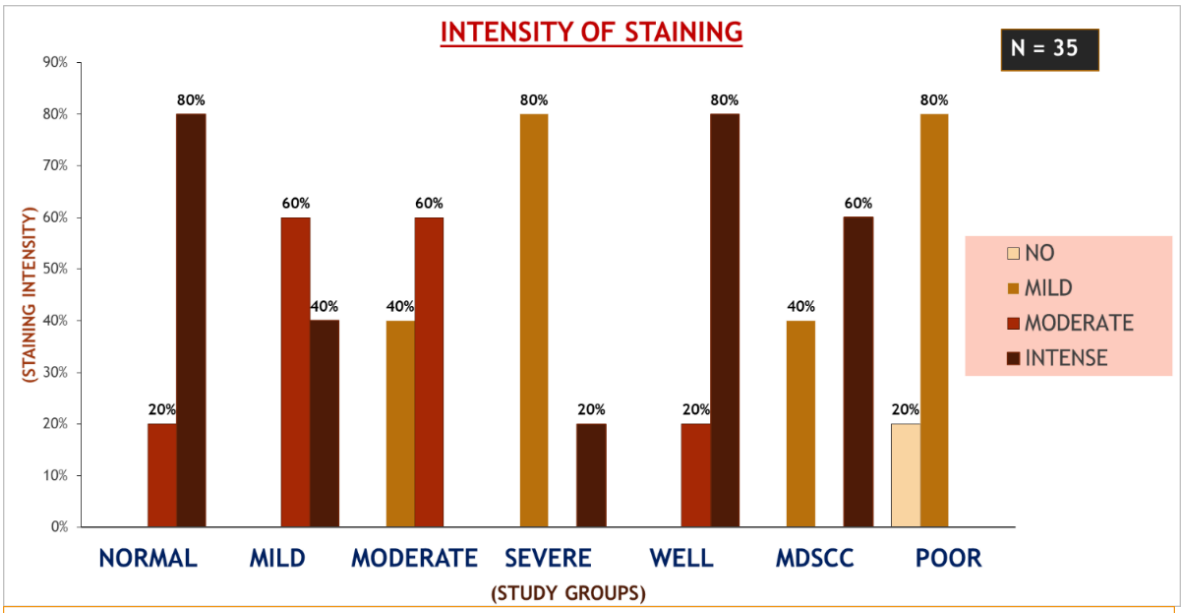
AREA OF STAINING TOTAL (N=15)	MILD (N =5)	MODERATE (N=5)	SEVERE (N=5)
1	0	1	4
2	0	2	0
3	4	2	0
4	1	0	1

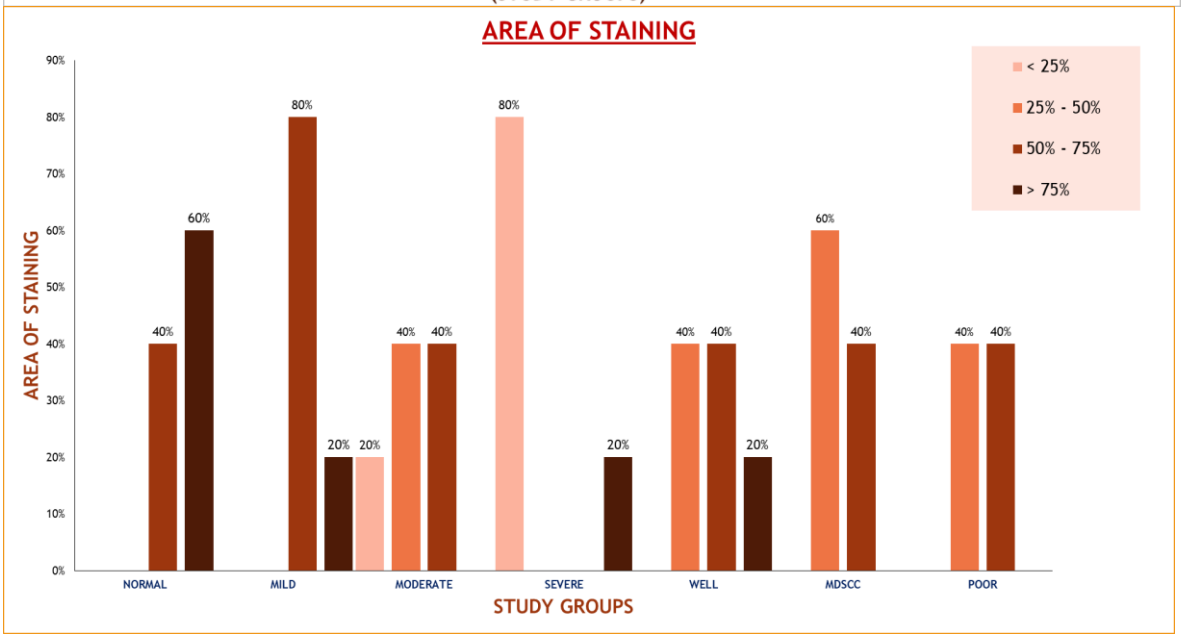
EXPRESSION OF E-CADHERIN IN ORAL SQUAMOUS CELL CARCINOMA:

(Group C) (TABLE 4)

INTENSITY OF STAINING TOTAL (N=15)	WELL DIFFERENTIATED OSCC (N =5)	MODERATELY DIFFERENTIATED OSCC (N=5)	POORLY DIFFERENTIATED OSCC (N =5)
0	0	0	1
+1	0	2	4
+2	1	0	0
+3	4	3	0

AREA OF STAINING TOTAL (N=15)	WELL DIFFERENTIATED OSCC (N=5)	MODERATELY DIFFERENTIATED OSCC (N=5)	POORLY DIFFERENTIATED OSCC (N=5)
1	0	0	1
2	2	3	2
3	2	2	2
4	1	0	0





AREA OF STAINING :
In normal mucosa (60%) 3 cases showed >75 of area stained and (40%) 2 cases showed 50-75% of area stained .
Out of 5 cases of mild dysplasia (20%) 1 case showed >75 % area stained and (80%) 4 cases showed 50-75% area stained. Among 5 cases of moderate dysplasia (40%) 2 cases showed 25-50% area stained, (40%) 2 cases showed 50-75% area stained and (20%)1 case showed <25% area stained .and out of 5 cases of severe dysplasia (20%) 1 case showed >75 % area stained and (80%) 4 cases showed <25% area stained .
Out of 5 cases of well differentiated OSCC (20%) 1 case showed >75 % area stained , (40%) 2 cases showed 50-75% area stained and (40%) 2 cases showed 25-50% area stained . Among 5 cases of moderately differentiate OSCC (40%) 2 cases showed 50-75% area stained, (60%) 3 cases showed 25-50% area stained and out of 5 cases of poorly differentiated OSCC (40%) 2 cases showed 50-75% area stained and (40%) 2 cases showed 5-50% area stained and 1 case showed absent or negative staining .
Statistically significant p value of 0.03 was obtained by kruskalwallis test. (Ttable 1, 2, 3, 4)

DISCUSSION:
Tumour markers are said to be biological or molecular attributes of tumour cells which distinguish them from normal cells, it is defined as a molecules that indicates the likely presence of cancer or one that provides information about the likely future behavior of an existing cancer. (16) Immunohistochemical studies is one of the most common as well as new molecular and genetic techniques used for better understanding of molecular insight of cancer for precise diagnosis and treatment planning. (8) Tumour markers act as a diagnostic aid and are useful in screening of early malignancies and determining the disease prognosis. (16) E-cadherin is one such tumour marker which plays an important role in epithelial cell adhesion, suppression of E- Cad is mainly responsible for dysfunction of cell-cell adhesion and total loss of its function leads to cancer progression. (15, 12) E-cad express are also studied in various carcinomas of head and neck region, prostrate, esophagus, pancreas and cervix. (8)
In the present study, it was observed that there was decreased E-cad expression in Oral OED with increase in grades of dysplasia and decreased expression of E-cad was also observed in OSCC when compared to normal epithelium.
The present study expression seems to be decrasing in intesity from well to poorly differentiated OSCC. These were in accordance with the study conducted by M.B.Yuwanati et al in 2011, and Kaur et al in 2009 and Monica et al in 2014 were they also concluded the reduced intensity of E-Cad with the severity of grades of OSCC. (12, 17, 18, 13)
Sridevi et al in 2015, studied and found decreased E-cadherin expression in dysplastic layer of epithelium which was in accordance with our study were mild membranous staining of E-cadherin in suprabasal and spinous layer of cells were observed. (8)
Hung et al in 2006, studied the immunoreactivity of E-cad expression and found decreased expression of OSCC. which was similar to our present study were progressively reduced expression of normal mucosa followed by oral epithelial dysplasia and significantly decreased in OSCC. (19)
As oral cancer is a multistep process requiring cell to cell modulation and cell to stromal interaction. As most of the malignancies have abnormality in cellular architecture and loss of tissue integrity, leading to local invasion of the tumour cells. E-cadherin expression has been reported to be hallmark of a cellular process kown as epithelial –mesenchymal transition (EMT), often implicated in cancer progression. There are multiple mechanisms that disrupts the E-cadherin function in cancer which includes inactivating somatic and germline mutations, epigenetic silencing by DNA methylation and epithelial to mesenchymal transition – inducing transcription factors and dysregulated protein processing. (15) The suppression of E-cadherin expression is considered as one of the main molecular events responsible for dysfunction of cell-cell adhesion and are correlated with increased invasiveness and metastasis of tumour, which also has a other name “supressor of invasion” gene. (13)
Our study was an attempt to study the expression of E-cadherin in dysplasias and OSCC and to understand the progression of dysplasias to OSCC. Currently there is no strong affirmation for the use of tumour markers in the progression of oral dysplasia and studies have proposed that LOH/A1, surviving, MMP9 positivity and DNA content are potential markers for increased opportunity of progression of oral dysplasia to cancer, other markers are p53, p73, MMP1, MMP2 and cathepsin L Mrna, Bcl-2, but did not predict progression. (9)

III. CONCLUSION:

From our study we conclude that absence or loss of E-cad expression cause alterations in epithelial characteristics of the cells which finally leads to higher invasiveness. Invasiveness of tumor cells can be assessed using an IHC panel of E-cad, which could help to predict tumor progression and prognosis. Tumor markers can be used for further research on the micro invasion of oral cancers and to facilitate early diagnosis and increase patient survival.

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