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Immunohistochemical expression of EP-CAM in different histopathological grades of oral squamous cell carcinoma

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Abstract

Background: oral squamous cell carcinoma(OSCC) is the most common type of head and neck cancer. The main etiological factor is found to be tobacco and other habits like betel nut, alcohol etc. Normal epithelial cell surface contains membrane glycoproteins which mediate cell to cell interaction and cellular recognition. Epithelial cell adhesion molecule(Ep-CAM) plays role in cell proliferation, invasion and metastasis. High Ep-CAM expression is found as the early finding in malignant transformation and can be used as marker for diagnostic purpose.

Aims and objective: Assessment of EP-CAM expression pattern and distribution in OSCC and normal oral mucosal tissue.

Material and method: The sample consisted of 40 cases and divided into three groups of different grades of OSCC and one group of normal oral mucosal tissue, ten in each group. All cases are immunohistochemically evaluated for the pattern and distribution of Ep-CAM using anti Ep-CAM antibody on the formalin fixed paraffin embedded tissue section by standardimmune histochemistry (IHC) technique and statistical analysis done.

Results: No statistical difference was obtained when the comparison was done in all four groups for pattern of expression and significant statistical difference was observed when comparison done for distribution of Ep-CAM.

Conclusion: The increase in Ep-CAM expression from normal mucosa to OSCC was observed, therefore Ep-CAM can be used for diagnostic or therapeutic purpose but further studies needed with large samples to establish same.

Keywords: OSCC, EP-CAM, IHC

Introduction

Approximately more than 95% malignant neoplasm of oral cavity is represented by oral squamous cell carcinoma (OSCC). The main etiological factor associated is tobacco, betel nut and consumption of alcohol.[1] OSCC being 13th in cancer morbidity and mortality in the world, so the early detection might improve patient survival and reduce treatment related morbidity.[2]

Epithelial cell adhesion molecule (Ep-CAM) is a calcium independent homophillic cell adhesion molecule.[3] Ep-CAM is encoded by GA733-2 gene.[4] It is expressed at lateral and basal domains of cell membranes and involved in cell to cell or cell matrix interactions in epithelium. Ep-CAM is found to be expressed on a variety of tumor cells playing role in cell proliferation, invasion and metastasis, which can be recognized by monoclonal antibodies and considered to be an important tumor marker and can be used for diagnostic and therapeutic purpose.[3]

To date many studies has been done on epithelial cancers as colorectal, breast, lung, hepatic carcinoma etc but only few studies on OSCC, hence the present study aims to assess the correlation of Ep-CAM expression in different grades of OSCC and normal oral mucosal tissue by IHC technique subsequent to early detection and help in planning therapeutic modalities at earliest, leading to better prognosis of the individuals.

Materials and Methods

The present study is carried out in department of oral pathology and microbiology, HKES's S. Nijalingappa institute of dental sciences and research Kalaburagi. Clinically and histopathologically OSCC confirmed cases of both genders with habits are included. Clinically and histologically confirmed normal oral mucosa were included in control group.

The total forty cases divided into group I (well differentiated) group II (moderately differentiated) group III (poorly differentiated) according to **BRODER's classification**[5] and group IV (normal oral mucosa as control group), ten in each group. All the specimens are routinely processed for H and E sections for the histopathological diagnosis and grading.

Immunohistochemistry

For IHC procedure the 4µm serial sections are obtained from the formalin fixed paraffin embedded tissue blocks on the poly- L lysine coated slides. The slides were deparaffinized and hydrated and the antigen retrieval is done using Concentrated antigen citra plus solution (Cat no.: HK080-9K; Biogenex Life Sciences Private Limited, CA, USA) by pressure cooker method. Enogenous peroxidase activity is blocked by treating the slides with the 3% hydrogen peroxide and power block for background staining. Then the sections were incubated with primary antibody (Monoclonal rabit anti-EpCam antibody Clone Number: E144; Biogenex Life Sciences Private Limited, CA, USA) at room temperature for 1 hour then Super Enhancer reagent for 20 min. Later the slides were incubated by secondary antibody (Super sensitive Polymer-horse radish peroxidase (HRP) immunohistochemical detection system.Biogenex Life Sciences Private Limited, CA, USA) for 20min and then substrate chromogen DAB (3,3-diaminobenzidinechromogen) solution is applied for 5min and counterstained by harris hematoxylin solution and mounted with DPX.

The same steps are followed for the positive control slides (adenocarcinoma of colon), while in negative control slides primary antibody is replaced by phosphate buffer solution (PBS).

Staining was assessed in two steps

a) **Assessment for pattern of the staining in the given section**

This was done under 100× using a compound microscope.

1. Patchy staining i.e. over staining at focal areas and other areas are homogenously stained was given value of 1.
2. Homogeneous staining was given value of 2.

b) **Assessment for distribution of the staining in the given section**

This was done under 400× using a compound microscope.

i. Cytoplasmic staining was scored +.

ii. Membranous staining was scored ++.

iii. Cytoplasmic and membranous combined was scored +++.

Statistical Analysis

The statistical analysis was done using statistical package for social science (SPSS for windows, version 16.0; SPSS INC., USA). The comparison between all the four groups for pattern and distribution expression of Ep-CAM was done using Chi-Square test. While, intergroup comparison for both pattern and distribution expression of Ep-CAM was done using fisher exact test. All the test were considered significant when the p value was less than 0.05 ($p<0.05$).

Results

The present study is immunohistochemically evaluated for Ep-CAM expression in control group and three histopathologically diagnosed different grades of oral sqamous cell carcinoma (OSCC). The OSCC is graded according to the **Broder's** Classification[5].

The study comprise of four different groups, each group consists of ten samples each.

Group 1: Well differentiated OSCC

Group2:ModeratelydifferentiatedOSCC

Group 3: Poorly differentiated OSCC

Group 4: Control group

In the present study, there are 8 males and 2 females in group 1, 9 males and 1 female in group 2, 7 males and 3 females in group3 and 7 males and 3 females in group 4(graph 1).

The mean age in group 1 is 40.2±9.21 with range of age varies from 30-55, mean age in group 2 is 44.3±10.44 with range of age varies from 28-65, mean age in group 3 is 51.3±6.86 with range of age varies from 38-60 and mean age in group 4 is 32.1±8.39 with range of age varies from 19-49 (graph 2).

Pattern of Ep-CAM expression in different groups are analyzed in two categories i.e. patchy and homogenous. In the group 1 the patchy pattern were 4 and homogenous pattern were 6, in group 2 the patchy pattern were 2 and homogenous were 8, in group 3 the patchy pattern were 5 and homogenous were 5 and in group 4 no patchy pattern observed whereas homogenous pattern were 10 (table 1 & graph 3). No statistical difference was obtained when the comparison was done in all four groups using Chi-square test (p-value>0.05)(table 1).

The positive immunostained areas showed distribution of Ep-CAM expression in cellular cytoplasm, on the membrane and in some cases in both the areas. The distribution of Ep-CAM expression in group 1 showed positive cytoplasmic immunostaining in 4 samples, membranous in 2 samples and in both areas in 4 samples, in group 2 the distribution of Ep-CAM expression in cytoplasm and membranous were observed in 3 samples each while for both areas 4 samples showed positivity, in group 3 the cytoplasmic distribution were observed in 2 samples, membranous was in one sample and in both areas were in 7 samples and in group 4 the cytoplasmic distribution were observed in 9 samples, membranous only in one sample(table 2 & Graph 4). Statistical significant difference was observed when the comparison were done between all different groups using Chi-square test (p-value<0.05) (table 2).

However, intergroup comparison was done using fisher's exact test shows statistical insignificant difference in the pattern of expression of Ep-CAM except for the comparison between poorly differentiated and control group where the comparison was statistically significant (table 3). Similarly, when comparison between groups were observed w.r.t. distribution in Ep-CAM expression also shows statistical insignificant difference.

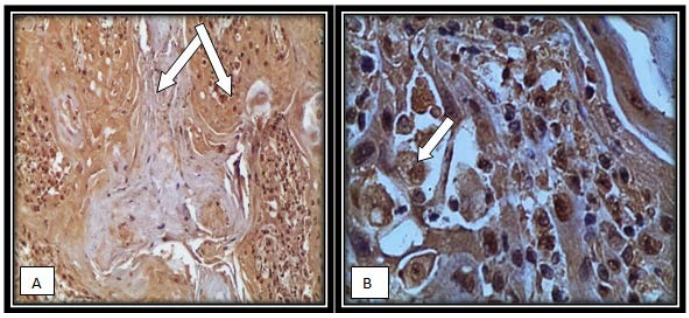


Figure 1: Ep-CAM Expression In Well Differentiated Oral Squamous Cell Carcinoma (A) PATCHY PATTERN (10X) (B) CYTOPLASMIC DISTRIBUTION (40X).

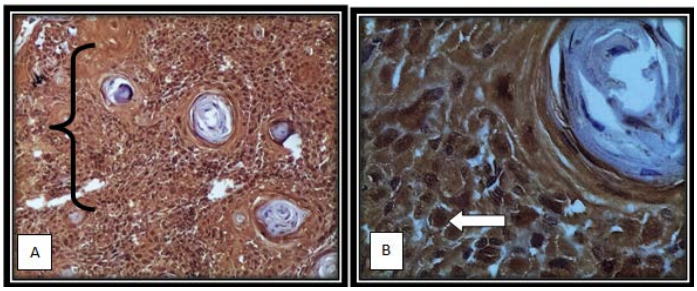


Figure 2 Ep-CAM expression in well differentiated oral squamous cell carcinoma (A) HOMOGENOUS PATTERN (10X) (B) CYTOPLASMIC& MEMBRANOUS DISTRIBUTION (40X)

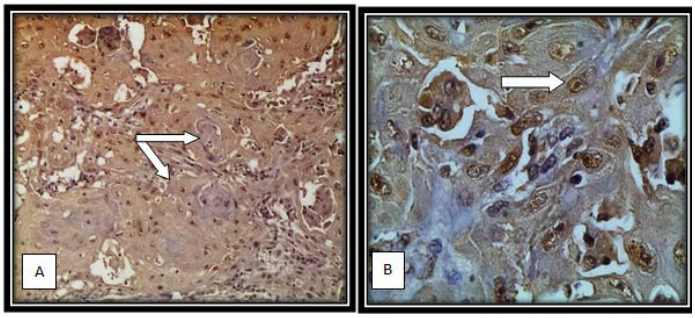


Figure 3: Ep-CAM expression in moderately differentiated oral squamous cell carcinoma (A) PATCHY PATTERN (10X) (B) MEMBRANOUS DISTRIBUTION (40X)

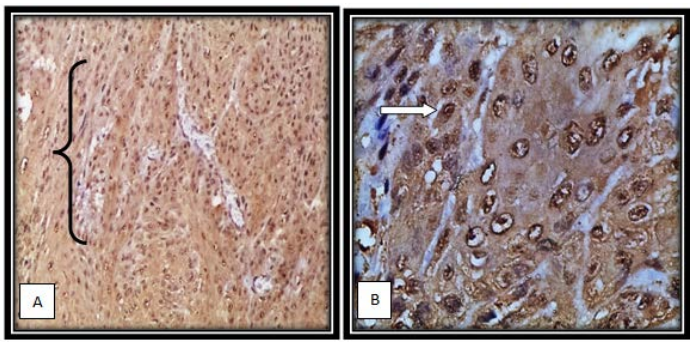


Figure4: Ep-CAM expression in poorly differentiated oral squamous cell carcinoma (A) HOMOGENOUS PATTERN (10X) (B) CYTOPLASMIC &MEMBRANOUS DISTRIBUTION (40X)

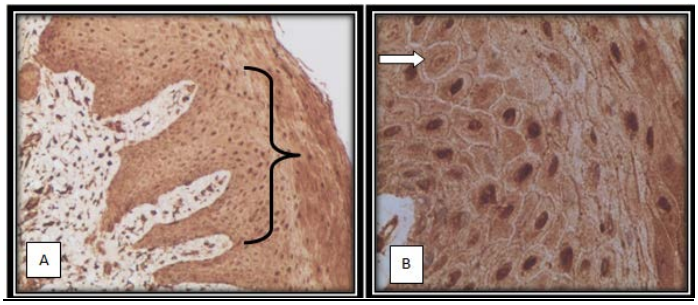
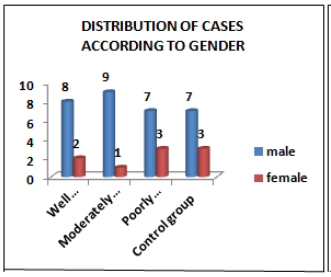
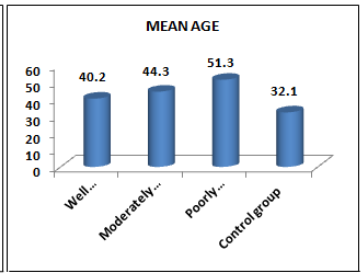


Figure 5: Ep-CAM expression in normal oral mucosal tissue (A) HOMOGENOUS PATTERN (10X) (B) CYTOPLASMIC DISTRIBUTION (40X)



Graph 1: Distribution of cases According to gender



Graph 2: mean age distribution in different groups

Table 1 : Pattern of ep-cam expression in different groups

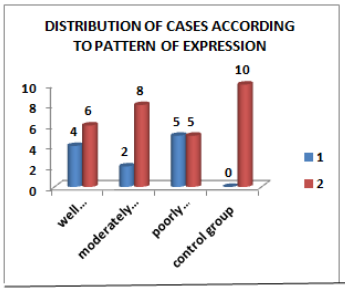
Serial No.	Groups	Patchy *	Homogenous**	Chi-Square value	p-value
1	Group I	4 (40%)	6 (60%)	7.39	0.0605 Non-significant
2	Group II	2 (20%)	8 (80%)		
3	Group III	5 (50%)	5 (50%)		
4	Group IV	0 (0%)	10 (10%)		

*score=1

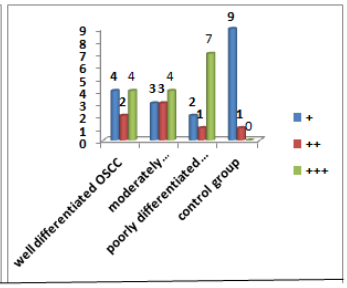
** score=2

Table 2: Type of ep-cam expression according to site specific (cytoplasmic/ membranous) in different groups

S.No.	Groups	Cytoplasmic (+)	Membranous (++)	Cytoplasmic & Membranous (+++)	Chi- Square test	P value
1	Group I	4 (40%)	2(20%)	4(40%)	14.62	0.0234 significant
2	Group II	3 (30%)	3(30%)	4(40%)		
3	Group III	2(20%)	1(10%)	7(70%)		
4	Group IV	9(90%)	1(10%)	0 (0%)		



Graph 3: Pattern of ep-cam expression in Different groups



Graph 4: Type of ep-cam expression according to site specific (Cytoplasmic/ Membranous) in different groups

Table 3: Comparison within different groups of ep-cam expression by fisher's exact test for pattern of expression and distribution

Serial No.	Groups	Pattern	Distribution
1	Group 1 & group 2	0.3847 **(Non-significant)**	0.8541 **(Non-significant)**
2	Group 1 & group 3	1.0000 **(Non-significant)**	0.0524 **(Non-significant)**
3	Group 1 & group 4	0.0867 **(Non-significant)**	0.22 **(Non-significant)**
4	Group 2 & group 3	0.3498 **(Non-significant)**	0.12 **(Non-significant)**
5	Group 2 & group 4	0.4737 **(Non-significant)**	0.44 **(Non-significant)**
6	Group 3& group 4	0.0325 **(Significant)**	0.58 **(Non-significant)**

Discussion

Cancers of the oral cavity is considered to be the 11th most common malignancy in the world leading to high mortality rate. Even though recent advances, survival rate is restricted to 5 years[6]. Thus it is necessitates one to identify the key marker which improve the early detection and the prognosis of the treatment. Over a period of time, Ep-CAM has been investigated for diagnostic and therapeatic purposes. Ep-CAM is a cell adhesion molecule (CAM), which mediates cell to cell adhesion, cell signaling, cell migration and proliferation. Overexpression of Ep-CAM protein has been demonstrated in various human carcinomas[7,8,9,10,11]. However, it is less explored in head and neck cancer. Although few studies had been done on Ep-Cam in oral squamous cell carcinoma but the studies about the pattern and distribution of expression of Ep-Cam in oral squamous cell carcinoma cases are very few.

In our present study we had immunohistochemically evaluated the pattern and distribution of the expression of Ep-CAM in ten cases of normal oral mucosal tissue and thirty oral squamous cell carcinoma cases, further which were classified according to the **Broder's classification**[5] into three groups namely, well differentiated, moderately differentiated and poorly differentiated. Each group of oral squamous cell carcinoma comprised of ten cases each.

The mean age of oral squamous cell carcinoma cases in our study is 45.26±10.23 and the age range varied from 28-65 years in which 20% are female and 80% are male (graph 1 and 2).

The present study showed the positive expression of cell surface antigen of Ep-CAM in all grades of oral squamous cell carcinoma. **Gupta et al[4],** whom observed the expression of Ep-CAM in all the grades of oral squamous cell carcinoma i.e. in the all thirty cases of their study group. They stated that in cancers there is degulation of Ep-CAM expression which controls the differentiation of the cells.

We observed different patterns of staining in oral squamous cell carcinoma i.e. patchy and homogenous staining of Ep-CAM. Analysing our study result we found that there is a decrease in patchy type pattern of expression of Ep-CAM from well differentiated to moderately differentiated oral squamous cell carcinoma (40% cases in group I, 20% cases in group II, Table-1) and again increased expression of Ep-CAM in poorly differentiated oral squamous cell carcinoma (50%

cases,Table-1) which is comparably low in comparison with the study of the results of **Gupta et al**[4],whom observed increase in patchy type of pattern as the disease advances (80%, 90% & 90% cases shows patchy type expression of Ep-CAM in three grades of OSCC respectively).

The **Shiah et al**[12] studies results were in contradiction to our results where, more homogenous type of pattern of expression of Ep-Cam in oral squamous cell carcinoma were reported. They were of the opinion that expression of Ep-CAM is an early event in tumorogenesis de novo. **High et al**[13] had also observed that homogenous pattern type of Ep-Cam expression is more in poorly differentiated oral squamous cell carcinoma (40% cases) then well and moderately differentiated oral squamous cell carcinoma which was again not in conformity of our results. They had stated that in keratinocytes, Ep-CAM expression is inversely correlated to squamous cell differentiation and is induced by cellular transformation,

We observed intense cytoplasmic staining of Ep-Cam in grade 1 which decreases as the grade of disease advances, (40% of cases in group I, 30% cases in group II and 20% cases in group III, Table 2) whereas the membranous staining of Ep-Cam increases from well differentiated oral squamous cell carcinoma to moderately differentiated oral squamous cell carcinoma (20% cases in group I, 30% cases in group II, Table 2) and reduces in poorly differentiated oral sqamous cell carcinoma(10% cases in group III). The expression of both cytoplasmic and membranous staining increases as disease advances (40% cases in group I, 40 % cases in group II & 70 % cases in group III, Table 2). These results are in accordance to the observations of **Gupta et al**[4]whom also observed increase in cytoplasmic staining of Ep-CAM and even the cytoplasmic and membrane staining of Ep-CAM was similar to our results**.** They stated that the cytoplasmic & membranous expression for cell adhesion is due to the cell surface glycoprotein(extracellular domain) interaction with intracellular cytoskeletal domain and any deletion in this cytoplasmic domain of Ep-CAM molecule will result in unstable adhesions and cause decrease cell aggregation which may enhance metastasis and invasion and from the primary tumour or carcinoma. We had observed an increase in membrane staining of Ep-Cam (30% in group II & 90% in group III) which was not in confirmatory to **Gupta et al**[4] results. They suggested that the cause for increase in membrane staining could be due to other origin, probably due to differentiation to either squamous or mesenchymal cell phenotype, thus expressed only on undifferentiated cells.

Although in our results we observed statistical insignificance when comparison was done within the grades of oral squamous cell carcinoma (p-value >0.05, Table 3) which is in contrary to **Gupta et al**[4] whom demonstrated a high statistical significance (p-value<0.01).

In the present study we had observed that the normal mucosa was positive for cytoplasmic or membranous staining (90 % cases shows cytoplasmic staining and 10 % showing membranous staining) which was inconsistence with the results of **Hwang et al**[14] whom reported that normal oral epithelial cells showed membranous and/or cytoplasmic Ep-CAM expression.

While our results were not in agreement with those of **Sen et al**[6] whom, observed no Ep-CAM expression in normal oral mucosal tissues.

In our study we had observed increase expression of Ep-CAM in oral squamous cell carcinoma cases than the control group cases (Table 1 & 2) , which was in accordance to the results of **Laimer et al**[1]studies, who showed increased Ep-CAM expression in oral squamous cancerous tissue compared to those in normal epithelium.

They suggested that in oral squamous cell carcinoma the Ep-CAM antigen appears to be expressed de novo. Their data indicated that Ep-CAM expression is linked to a more aggressive type of cancer. They concluded that a tumor promoting effect due to overexpression of Ep-CAM results in increased migration, proliferation and metastasising potential of tumor cells. They also stated that the patients presenting Ep-CAM overexpression would qualify for Ep-CAM directed immunotherapeutic approaches.

But our results was contrary to results (Table 1 & 2) of **Hwang et al**[14] who had observed a progressive reduction in the expression of Ep-CAM from normal to dysplastic to cancerous oral epithelium and suggested that high areca nut consumption in those geographic areas resulted in this variation. They also postulated that arecanut will induce gingival keratinocytes for the production of tumour necrosis factor α which downregulates Ep-CAM expression. Thus this protein is indicated as an important event in the development of oral cancer.

Conclusion

The present study had evaluated the pattern and distribution expression of Ep-Cam by immunohistochemical methods. In this study it is observed that over expression of Ep-Cam was localized to tumour cells and over expression of patchy type of pattern as the disease advances. The present study had also demonstrated the increase in cytoplasmic and/or membrane Ep-Cam expression from normal oral mucosal tissue to oral squamous cell carcinoma. This signifies the role of Ep-Cam in carcinogenesis of squamous cell carcinoma of oral cavity. Therefore, Ep-Cam can be used as diagnostic and therapeutic marker for oral squamous cell carcinoma. Further studies with larger samples are required to establish its confirmatory role in oral squamous cell carcinoma.

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