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Original Research

Expression of Epithelial Glycoprotein (EGP40) in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma (OSCC)

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Abstract

Aim: To study the expression of Epithelial Glycoprotein (EGP40) in various grades of Oral Epithelial Dysplasia namely mild, moderate and severe epithelial dysplasia and in various grades of Oral Squamous Cell Carcinoma (OSCC) namely well, moderate & in poorly differentiated squamous cell carcinoma. **Material and Methods:** This laboratory study comprised of 47 samples in which 27 samples were of three different grades of OSCC, Well (*n*=10), Moderate (*n*=10) and Poorly differentiated (*n*=7) and remaining 20 samples were of three different grades of dysplasia [Mild (*n*=7), Moderate (*n*=8), Severe dysplasia (*n*=5)]. All the 47 cases were subjected to immunohistochemical staining to evaluate the expression of EGP40. Chi square was used to analyze the significance of differences in different grades of oral epithelial dysplasia & in different grades of OSCC. **Results:** The percentage of EGP40 immuno positivity was increased from mild to severe dysplasia and the percentage of EGP40 immuno positivity was increased from well to poorly differentiated carcinoma No statistical significance was observed in the immunoreactivity and in the intensity of expression between various grades of oral epithelial dysplasia and oral squamous cell carcinoma. **Conclusion:** The epithelial cell adhesion molecule can be considered as an early marker for predicting malignant transformation of dysplastic epithelial lesions and used as a marker in predicting the OSCC prognosis.

Keywords: *EGP40, oral potentially malignant, oral squamous cell carcinoma*

Introduction

Leukoplakia is one of the common potentially malignant disorder of the oral cavity.[1,2] Leukoplakia is defined as " a white lesion of the oral mucosa that cannot be characterized clinically or microscopically as any other defined oral disease entity".[3,4] The global prevalence of oral leukoplakia range from 0.5% to 3.46%, and the carcinomatous transformation of oral leukoplakia range from 0.7% to 2.9%.[5] Oral leukoplakia is more prevalent in India, where the habit of tobacco and areca nut chewing and smoking is more than elsewhere.[6] As the grades of epithelial dysplasia progress from mild to severe, there is an increased risk of an individual leukoplakic lesion to progress to carcinoma.[7-10] Cancer is one of the most common causes of morbidity and mortality. Oral cancer is the sixth most common cancer in the world.[11,12]

Cell adhesion is a fundamental process required for the correct functioning of multicellular organisms. They are involved in a broad range of processes, including cell to cell and cell to matrix interactions, cell migration, cell cycle, and signalling as well as in morphogenesis during development and tissue regeneration. Cell Adhesion Molecules (CAMs) are involved in a variety of pathologies ranging from cancer, inflammation, pathogenic infections to autoimmune diseases.[13] Four major families of CAMs are recognized on the basis of their structure: integrins, selectins, CAMs of the immunoglobulin gene (IgG-like) superfamily, and cadherins.[14] Epithelial cell adhesion molecule (EpCAM) is one of the CAMs exist that does not share any of the structural patterns of the four CAM families.[13]

EpCAM was initially described as a tumor-associated antigen by Koprowski and colleagues in 1979.[13] The EpCAM is also called EGP40, ESA, KSA, 17-1 A antigen and they function as a homotypic intercellular adhesion molecule.[15] EGP40 is a 40-kDa epithelial transmembrane glycoprotein encoded by the GA733-2 gene, which is located on chromosome 4q.[16]

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It is usually expressed at the basolateral membrane of cells by the majority of simple cuboidal or columnar, pseudostratified columnar, and transitional epithelia but is absent in squamous epithelium and in some specific epithelium cell types, such as epidermal keratinocytes, gastric parietal cells, thymic cortical epithelium, myoepithelial cells, and hepatocytes.[14,17] EpCAM is not expressed on tumors of mesodermal origin.[13] The EpCAM is considered as an early marker for potentially malignant and malignant lesions, due to its high expression on rapidly proliferating tumors of epithelial origin**.** EpCAM overexpression usually correlates with a decrease in survival rate.[13]

The aim of this study is to study the expression of EGP 40 in oral epithelial dysplasia, which is clinically diagnosed as leukoplakia and in oral squamous cell carcinoma (OSCC) and to compare the expression of EGP 40 between different grades of epithelial dysplasia (mild, moderate, severe epithelial dysplasia) as well as in different grades of OSCC (well, moderate and poorly differentiated OSCC).

Methods and Materials

This laboratory study comprised a total of 47 cases, of which 20 cases of oral epithelial dysplasia (Group I) and 27 cases of OSCC (Group II) were evaluated for EGP40 expression.

Among 20 cases of oral epithelial dysplasia (Group I) EGP40 expression was assessed under three subgroups, namely mild dysplasia (n = 7), moderate dysplasia (n = 8), and severe dysplasia (n = 5).

Similarly, among a total of 27 cases of OSCC (Group II) EGP40 expression was assessed under three subgroups, which were graded according to Broder's histopathological grading system namely well-differentiated (n = 10), moderately differentiated (n = 10), and poorly differentiated (n = 7) OSCC. The distribution of cases is presented in Table 1.

All the cases of oral epithelial dysplasia and the OSCC were taken from the archives of the Oral and Maxillofacial Pathology department of our Institute.

These archival tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax, two sections each of 4 µm thickness were prepared in rotary soft tissue microtome (Thermo Scientific HM 340E) for doing hematoxylin and eosin stain and immunohistochemical staining. For immunohistochemistry, tissue sections were taken on 3-amino propyl triethoxy silane (APES) precoated slides. Prostate tissue was used as a positive control in this study.

All the study samples were subjected to immunohistochemical staining to evaluate the expression of EGP40. The sections were deparaffinized and rehydrated in graded alcohol. Sections were then treated with trypsin stabilized solution (concentrated) and trypsin stabilized solution (Buffer) (Scy Tek Laboratories- USA) in the ratio of 1:3 for 20 minutes at room temperature for retrieving the antigen.

All the reagents were brought to room temperature before immunostaining. All the incubations were performed at room temperature by using a humidifying chamber. The sections were covered with 3% hydrogen peroxide for 10 minutes, following which it was treated with power block (Scy Tek Laboratories- USA) for 10 minutes to avoid cross-reactions.

The sections were covered with primary antibody EGP40 (Thermo Scientific - USA) at 37°C temperature for 45 minutes. The slides were washed and treated with secondary antibody tagged with poly horseradish peroxidase enzyme (HRP) for 30 min (Scy Tek Laboratories – USA) followed by treating the sections with freshly prepared 3, 3-diaminobenzidene tetra hydrochloride (DAB) solution for 5 minutes. The slides were immersed in Mayer's hematoxylin for 7 minutes and were dehydrated in graded alcohol air dried, cleared with xylene and mounted using dibutyl pthalate xylene (DPX).

Interpretation of staining

The presence of brown color end product was considered a positive immunoreactivity. The intensity of the expression was evaluated as mild, moderate, and intense in both oral epithelial dysplasia and in OSCC [Figures 1 and 2].

Statistical analysis

All the parameters were tabulated and assessed for statistical significance using Statistical Package for Social Science (SPSS) software version 17. The differences in the EGP40 expression in and between the different grades of oral epithelial dysplasia and in and between different grades of OSCC were statistically analyzed using the Chi-square test. The level of significance ($P < 0.05$) was employed in all statistical comparisons.

Table 1: Distribution of cases among the two study groups

Groups	Total no of cases	Number of cases		
Oral epithelial dysplasia (Group I)	20	Mild dysplasia 7	Moderate dysplasia 8	Severe dysplasia 5
Oral squamous cell carcinoma (Group II)	27	Well Differentiated 10	Moderately differentiated 10	Poorly differentiated 7

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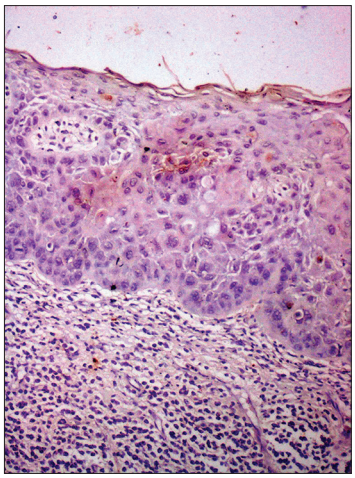


Figure 1: Immunohistochemical expression of EGP40 in oral epithelial dysplasia [x20X]

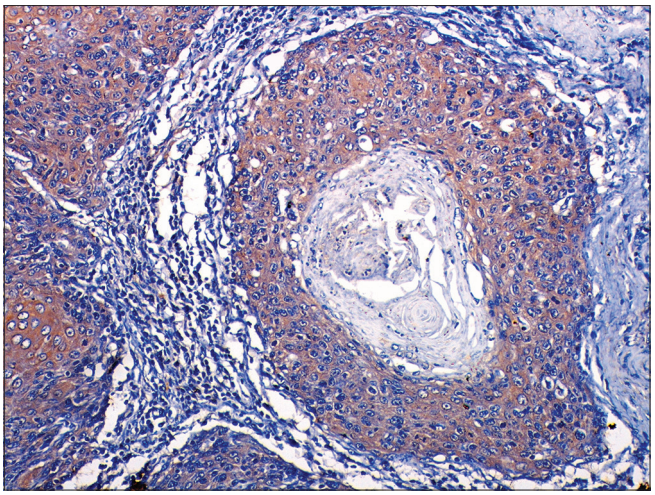


Figure 2: Immunohistochemical expression of EGP40 in oral squamous cell carcinoma [x20X]

Results

Among different grades of oral epithelial dysplasia, the percentage of immunopositivity of EGP40 was gradually increased from mild to severe dysplasia (mild dysplasia: 42.9%, moderate dysplasia: 50%, and severe dysplasia: 60%) [Table 2].

Among different grades of OSCC, the percentage of immunopositivity of EGP40 was gradually increased from well to poorly differentiated carcinoma (well differentiated OSCC: 40%, moderately differentiated OSCC: 60%, and in poorly differentiated OSCC: 71.5%) [Table 3].

No statistically significant difference was observed in different grades of oral epithelial dysplasia and in different grades of OSCC.

Table 2: EGP40 expression in mild, moderate and severe Oral Epithelial Dysplasia

EGP40 expression (Staining intensity)	No of cases	Percentage of cases
a: EGP40 expression in mild epithelial dysplasia		
Negative	4	57.1%
Mild expression	3	42.9%
Moderate expression	0	0%
b: EGP40 expression in moderate epithelial dysplasia		
Negative	4	50%
Mild expression	3	37.5%
Moderate expression	1	12.5%
c: EGP40 expression in severe epithelial dysplasia		
Negative	2	40%
Mild expression	3	60%
Moderate expression	0	0%

Table 3: EGP40 expression in well, moderate and poorly differentiated OSCC

EGP40 expression (Staining intensity)	No of cases	Percentage of cases
a: EGP40 expression in well-differentiated squamous cell carcinoma group		
Negative	6	60%
Mild expression	3	30%
Moderate expression	1	10%
b: EGP40 expression in moderately differentiated squamous cell carcinoma group		
Negative	4	40%
Mild expression	3	30%
Moderate expression	3	30%
c: EGP40 expression in poorly differentiated squamous cell carcinoma group		
Negative	2	28.6%
Mild expression	3	42.9%
Moderate expression	2	28.6%

Discussion

EGP40 is a transmembrane glycoprotein mediating Ca^{2+} independent homotypic cell to cell adhesion in the epithelium.[17] It is also involved in cell signaling, migration proliferation, and differentiation.[18,19]

EpCAM expression is observed in various tumorous conditions like in breast carcinoma, epithelial ovarian carcinoma, gall bladder carcinoma, cholangiocarcinoma, ampullary pancreas carcinoma, SCC of esophagus, SCC of head and neck region, gastric carcinoma, clear cell renal carcinoma, colon carcinoma, and in non-small-cell lung carcinoma.[20]

In this study, 10 out of 20 cases of oral epithelial dysplasia showed immunopositivity for EGP40 and the total percentage of positivity is 50%.

High AS *et al.*, in 1996 studied 22 cases of oral epithelial dysplasia in which all the cases were positive for EGP40 expression. This is not in agreement with our study where only 50% of cases were positive. The decrease in

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expression in this study may be due to inadequate antigen unmasking.[21]

In the subgroups of oral epithelial dysplasia, the expression of EGP40 showed 42.9% immunopositivity in mild dysplasia, 50% immunopositivity in moderate dysplasia, and 60% immunopositivity in severe dysplasia. However, no statistical significance among different grades of oral epithelial dysplasia was found, which may be due to limitations in our sample size.

Most of the cases found by High *et al*., belong to the category of mild/moderate dysplasia (17 cases) and 5 cases of severe dysplasia. It may be observed that High *et al*. have not differentiated between mild and moderate dysplasia. Other than the study of High *et al*. no other study on EGP40 expression in oral epithelial dysplasia is available for comparison.

In this study, 15 (56%) out of 27 cases of OSCC showed immunopositivity for EGP40. The study done by Went *et al*., in 2004 stated that out of 49 cases, 29 (59%) cases showed immunopositivity of EGP40.[16] Similarly, the study done by Spizzo G *et al*., in 2011 stated that out of 126 cases, 75 (59.5%) cases showed immunopositivity of EGP40.[22] The results of our study showed 56% immunopositivity for EGP40 is in agreement with Went *et al*., (59%) and Spizzo G *et al*., (59.5%).

The study done by Gupta V and Rao NN in 2011 showed 100% immunopositivity by scoring the staining pattern (patchy or homogenous) and the distribution of staining (cytoplasmic or membranous).[23] The percentage of total immunopositivity observed by Gupta V and Rao NN in 2011 varies from that of our study and previous studies of Went *et al*., in 2004 and Spizzo G *et al*., in 2011.

In 2009, Shiah *et al*., have shown that EGP40 expression was heterogeneous. The heterogeneous expression of EGP40 due to its dualistic role may help in preventing the metastasis or promote tumor invasion and metastasis depending upon the tumor microenvironment.[24] Such heterogeneous expression may account for lesser positive expression in this study and other studies by Went *et al*., and Spizzo G *et al*., when compared to Gupta V and Rao NN.

In our study, in the subgroups of OSCC, the expression of EGP40 showed 40% immunopositivity in well-differentiated SCC, 60% immunopositivity in moderately differentiated SCC, and 71.5% immunopositivity in poorly differentiated SCC. However, no statistical significance among different grades of OSCC was found, which may be due to limitations in our sample size**.** Our results showed that the percentage of positivity was seen to be increased as the grades increased from well-differentiated (40%) to poorly differentiated SCC (71.5%).

The role of EGP40 in cancer progression can be correlated with the ability of its direct impact on cell cycle and proliferation. It disrupts the cellular adhesion by abrogating E-cadherin- mediated cell adhesion by disrupting the link between α-catenin and F-actin, which points to possible involvement of EGP40 in tumor progression by enabling the cell migration, metastasis, and invasion.

EGP40 also has a role in cell motility and become involved in signal transduction and cell proliferation via upregulation of cyclin A and cyclin E synthesis. Finally, EGP40 also induces the upregulation of the proto-oncogene c-myc.[14,25-27]

Earlier studies have stated that the increase in EGP40 expression in OSCC was correlated with the upregulation of proto-oncogene, c-myc.[28] and cell cycle regulating genes cyclin A and E.[27] Its expression results in the activation of several genes that are involved in growth regulation and differentiation.[29]

Although a direct comparison between EGP40 expression in oral epithelial dysplasia and in OSCC cannot be meaningfully made, we did notice that the percentage of positivity in well-differentiated SCC was lesser than in moderate and severe dysplasias. This could probably be due to the different roles played by EGP40 in different tumor microenvironments. It is thought to mediate cell-to-cell adhesion and prevent cell scattering. It also plays a role in tumor invasion and metastasis. It is probable that EGP40, while aiding in an epithelial invasion into connective tissue, also tends to maintain cell cohesiveness, which is a histological feature of well-differentiated SCCs. As we progress to moderate and poorly differentiated SCCs, the EGP40 expression was seen to proportionately increase, which again correlates with the histological picture of lack of cohesiveness in such grades of carcinomas.

Fong *et al*., in 2006, reviewed the expression of EpCAM in SCC of head and neck and had stated that its overexpression has a strong negative impact on overall survival.[30]

This study, which showed a steady increase in expression from well-differentiated to poorly differentiated SCC may be linked with poor prognosis and thereby overall survival; is thus in agreement with Fong *et al*. Therefore, EGP40 may act as a potential prognostic marker and a potential target for immunotherapeutic strategies.

The sample size of this study though small gives an insight into the expression of EGP40 in different grades of OSCC and in different grades of oral epithelial dysplasia. Hence, larger samples are required to establish unequivocally the role of EGP40 in early molecular interaction which is critical in the initiation and progression of the tumor. It may also help us to understand the mechanism of metastatic behavior, and thus aid in the design of new strategies for diagnosis and treatment of OSCC.

Conclusions

In this study, the percentage of positivity of EGP40 expression was increased as the grades of oral epithelial

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dysplasia progressed from mild to severe dysplasia. Similarly, the percentage of positivity of EGP40 expression was increased as the grades of OSCC increased from well-differentiated to poorly differentiated carcinoma thus suggesting its usefulness as an early marker in assessment for premalignancies and as an indicator of the progression of the tumor, thereby predicting the prognosis.

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Conflicts of interest

There are no conflicts of interest.

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