Original Article

Expression of cluster differentiation-44 stem cell marker in grades of oral epithelial dysplasia: A preliminary study

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Abstract

Introduction: Oral cancer is one among the alarming diseases related to oral cavity. Its prevalence and incidence have increased in many folds, in the past decade. This has led the investigators to find the preliminary stages and related early evaluating methods to restrain it. Few clinical lesions such as leukoplakia, erythroplakia, oral submucous fibrosis and lichen planus reflected malignant changes. These premalignant disorders provided scope to assess the underlying cellular and molecular events, which shall be helpful in early detection, aggressiveness and prognosis of the patient.

Materials and Methods: Forty formalin fixed, paraffin embedded blocks were utilized and evenly subdivided into Group I – control tissue, Group II – mild epithelial dysplasia, Group III – moderate epithelial dysplasia and Group IV – severe epithelial dysplasia. The study group was categorized based on the WHO classification of dysplasia 2005. Routine staining was performed to reconfirm the diagnosis of all the samples. Simultaneously, immunohistochemical staining was done with cluster differentiation-44 (CD44) antibody. Positive cells were counted on 10 representative fields with a minimum of 100 cells per field using ×20.

Statistical Analysis: Comparison of four groups with respective to number of positive cells was done using Kruskal–Wallis ANOVA test. Pair-wise comparison of three grades of oral epithelial dysplasia and the controls was done using Mann–Whitney U test.

Results: The mean of Group I is 745.50, Group II is 665.20, Group III is 530.10 and Group IV is 322.90. A statistically significant $P = 0.00001$ was ascertained on comparison of the mean between the groups.

Conclusion: CD44, a cell membrane marker could help in cell adhesion and cell-cell interactions. Loss of CD44 expression enhances the binding of the growth factors with their principle receptors that enhances the cellular proliferation. It can be used as a prognostic marker for identifying the rate of malignant transformation in these disorders.

Keywords: CD44, cell membrane marker, dyaplasia, stem cell

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INTRODUCTION

Oral cancer is a major health problem in many parts of the world. While the incidence is relatively low in western countries, it remains to be one of the most common forms of cancer, in the Indian subcontinent and other parts of Asia.[1] Its strong correlation with specific risk factors, such as tobacco and alcohol use cause genetic damage. Thus, leading to uncontrolled proliferation of these cells resulting in dysplasia and presents as precancer and cancer.[2]

Oral leukoplakia represents the most common potentially malignant disorder of the oral cavity. Worldwide prevalence of leukoplakia is 0.2%–4.9% and the overall malignant transformation rates for dysplastic lesions range from 3% to 6%, depending on the type, size of the lesion and length of follow-up. The presence of epithelial dysplasia histopathologically may be even more important in predicting malignant potential than the clinical characteristics.[3]

Dysplasia is a Greek word meaning abnormal atypical proliferation of tissues. The term *"dysplasia"* was introduced by Reagon in 1958 in relation to the cells exfoliated from lesions of the uterine cervix. Dysplasia is encountered principally in the epithelium. In the past, epithelial dysplasia, epithelial atypia and dyskeratosis were used synonymously.[4]

Oral epithelial dysplasia (OED) is the diagnostic term used to describe the histopathologic changes seen in a chronic, progressive and premalignant disorder of the oral mucosa. OED is not associated with any specific clinical appearance. However, leukoplakia and erythroplakia are the lesions classically associated with dysplastic changes. It is also consistently seen in the mucosa adjacent to the tumor in patients with invasive squamous cell carcinoma.[5]

Various markers such as Ki-67, p53, proliferating cell nuclear antigen, argyrophilic nucleolar organizer region and cytokeratins and notch genes have been utilized to identify OED that will progress to malignancy.[6]

In the past few years, there has been increasing interest in family of surface glycoproteins called cluster differentiation-44 (CD44). These are first described in 1983 as lymphocyte homing receptor. It is encoded by a single gene containing 20 exons located on chromosome 11p13. CD44 was originally implicated as a hyaluronic acid and a homing receptor directing the migration of circulating lymphocytes across the high endothelial venular membranes of the lymph nodes and inflamed synovia.[7]

CD44 is not only expressed in lymphocytes but also a wide variety of epithelial tissues. Functionally speaking, CD44 is involved in organ integrity through its ability to contact extracellular matrix. It serves as a co-receptor for numerous transmembrane proteins such as matrix metalloproteases, members of the ERB family of receptor tyrosine kinases, and the long known tumor-associated antigens EpCAM (CD326, ESA1).[8]

CD44 was in the focus of molecular oncology in the early 1990s when it was recognized that variants of it, chiefly CD44 v6, regulate tumor progression, invasion and metastasis formation.[9] The nature of this transmembrane adhesion molecule and the role it plays in the tumor development and progression are matters which have interested not only the basic researchers but also cancer clinicians and pathologists. The availability of different exon-specific monoclonal antibodies against CD44 variants has enhanced the ease and accuracy of immunohistochemical analysis. However, there have been comparatively few studies involving CD44 expression and premalignant oral lesions.[10]

The current study aims to evaluate the immunohistochemical expression of CD44 in OED and whether it can serve as a prognostic marker.

MATERIALS AND METHODS

The present study was carried after the ethical clearance from the Institutional Review Board (Protocol no.22/IEC-SIBAR/17) on 24th December 2017. A total of 40 samples were utilized. Formalin-fixed, paraffin embedded blocks, categorized according to 2005 WHO classification of dysplasia were included in the study. Apparently normal healthy oral mucosa without any of the clinically obvious lesions was included in the control group. Those patients who are having a habit of smoking, drinking alcohol and chewing are excluded. The study sample was divided into Group I – control tissue (10), Group II – mild epithelial dysplasia (10), Group III – moderate epithelial dysplasia (10) and Group IV – severe epithelial dysplasia (10).

Serial sections of 4 μ were obtained from the archival material. The sections of all the samples were first subjected for routine hematoxylin and eosin examination to reconfirm the diagnosis. Later, other sections of all the four groups were subjected for immunohistochemical analysis using CD44 antibody. The positive CD44 expression was seen as a light brown stain in the cells of the epithelium [Figures 1-4]. These cells were counted on 10 representative fields with a minimum of 100 cells per field using ×20.

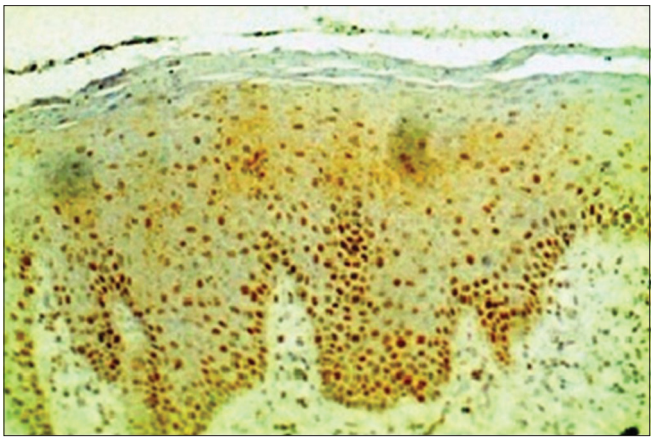

Figure 1: Cluster differentiation-44 immunopositivity of normal mucosa, ×10

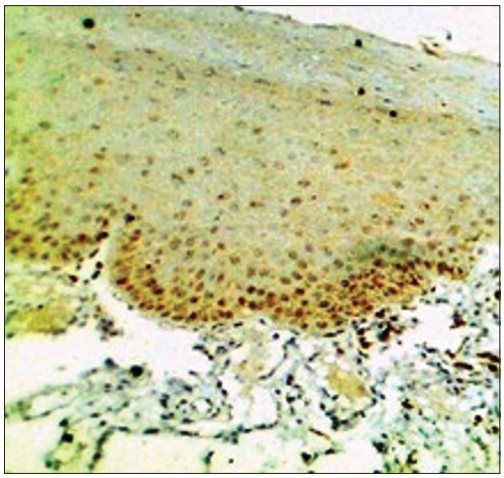

Figure 2: Cluster differentiation-44 immunopositivity of mild epithelial dysplasia, ×10

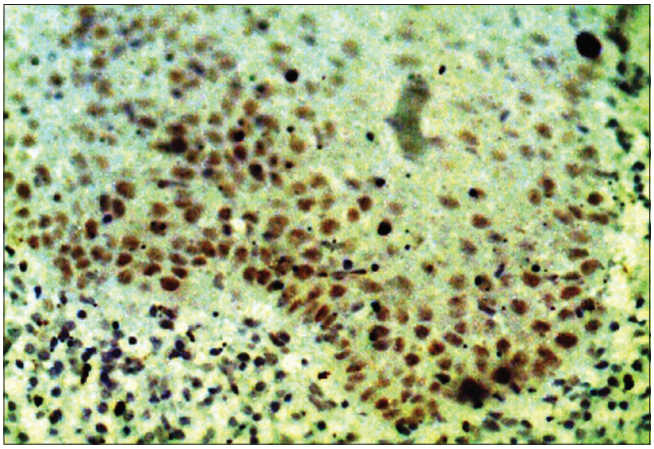

Figure 3: Cluster differentiation-44 immunopositivity of moderate epithelial dysplasia, ×20

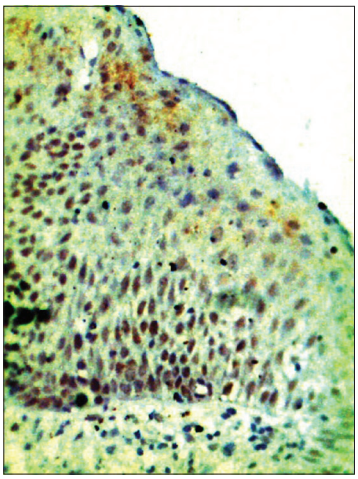

Figure 4: Cluster differentiation-44 immunopositivity of severe epithelial dysplasia, ×20

Research microscope BX51 manufactured by Olympus, a jenoptik CCD camera and image analyzer software (Pro Express) were utilized.

Statistical analysis

The collected data were entered into the Excel sheet, and statistical analysis was done using software, Statistical Package for Social Sciences version 20.0 (IBM Bussiness Corporation, Chicago, USA). The comparison of four groups with respective to number of positive cells was done using Kruskal–Wallis ANOVA test. Pair-wise comparison of three grades of OED and the controls was done using Mann–Whitney U test.

RESULTS

The mean and standard deviation of each group was determined Kruskal–Wallis ANOVA. The mean of Group I is 745.50, Group II is 665.20, Group III is 530.10 and Group IV is 322.90. The standard deviation of four groups is 68.17, 68.94, 56.69 and 42.77, respectively. A statistically significant $P = 0.00001$ was ascertained on comparison of the mean between the groups, as shown in Table 1.

Pair-wise comparison of four groups with respect to number of cells was done using Mann–Whitney U-test. A significant *P* value was noticed on the comparison between the groups [Table 2].

DISCUSSION

Oral precancerous lesions are altered epithelial lesions which have an increased likely hood to progress toward oral squamous cell carcinoma. Recently, the term premalignant lesions and conditions have been replaced with a common favorable terminology "potentially malignant disorder."[11]

Most common potentially malignant disorders of the oral cavity are leukoplakia and erythroplakia. Around 50% of the oral squamous cell carcinoma arises from these lesions.

Table 1: Comparison of four groups with respect to number of cells by Kruskal-Wallis ANOVA test

Groups	Mean	SD	*P*
Group I	745.50	68.17	0.00001*
Group II	665.20	68.94	
Group III	530.10	56.69	
Group IV	322.90	42.77	

*$P<0.005$. SD: Standard deviation

Table 2: Pair-wise comparison of four groups with respect to number of cells by Mann-Whitney U-test

Groups	Mean	SD	*Z*	*P*
Group I	745.50	68.17	−2.2678	0.0233*
Group II	665.20	68.94		
Group I	745.50	68.17	−3.7041	0.0002*
Group III	530.10	56.69		
Group I	745.50	68.17	−3.7796	0.0002*
Group IV	322.90	42.77		
Group II	665.20	68.94	−3.3639	0.0008*
Group III	530.10	56.69		
Group II	665.20	68.94	−3.7796	0.0002*
Group IV	322.90	42.77		
Group III	530.10	56.69		
Group IV	322.90	42.77	−3.7796	0.0002*

*$P<0.005$. SD: Standard deviation

The potentially malignant lesions can be assessed using hematoxylin and eosin for the architectural and cytological changes which is generally referred as epithelial dysplasia.[12]

The specific alterations of the epithelial cells are important to determine dysplastic cells. The nuclei take a more primitive appearance, similar to those of basal cells, often show nuclear enlargement, dark-staining nuclei and an increased nuclear-to-cytoplasmic ratio as well as variation in the shape of the cells and nuclei. These are unusual outside cancers and precancers.[13]

The 14th International Cancer Congress in Hungary suggested the following microscopic changes for the diagnosis of OED: drop-shaped rete processes, disturbed nuclear polarity, basal cell hyperplasia, disturbed epithelial maturation, pleomorphic cells, anisocytosis, hyperchromatic nuclei, prominent nucleoli, increased nuclear-cytoplasmic ratio, cell crowding, increased number of mitoses, abnormal mitoses and reduced cellular cohesion.[14]

The abnormalities in the nuclear morphology are frequently seen in the dysplasia such as the nuclear diameter, shape, nuclear area, number of nucleoli and membrane outline. This can be correlated with the rate of malignant transformation and prognosis of the disease.[15] The oral epithelial dysplasias are more likely to progress into cancer. The actual mechanism of progression is poorly understood, and there is no evidence that a dysplastic lesion will surely progress into cancer.[16]

Grading of OED's will help us to lower the inter- and intra-observer variation, and it give us a clear separation between the patients who need treatment to prevent malignancy.[17] There are several grading systems followed to grade OEDs such as Shafer's (1993), Neville (1995), Ljubljana (2003) and WHO (2005). Among these most commonly followed is the WHO system, in which it is divided into three grades such as mild, moderate and severe epithelial dysplasia. The WHO grading system was considered in the present study which is universally accepted. These grading processes are basically structured on the potential risk for malignant transformation showing a range from 3% to 6%.[18]

There are several methods for identification, but the gold standard of identification is tissue biopsy. Molecular markers with immunohistochemical procedures can determine the prognostic features and rate of malignant transformation.[19]

Stem cells constitute a distinct subset of cells characterized by their capacity to self-renewal and differentiation into multilineage cellular constituents of a specific tissue or organ.[20]

The stem cells are of different types such as embryonic stem cells, adult stem cells and cancer stem cells. The embryonic stem cells are isolated from the inner cell mass of the blastocyst are pluripotent and can differentiate into three germ layers (ectoderm, endoderm and mesoderm). Adult stem cells are found in various adult tissues, which are typically more limited to with respect to differentiation, they are considered as multioligopotent.[21]

Cancer stem cells are a small subpopulation of cancer cells that have a unique ability of self-renewal and are potent to differentiate into progenitor cells. The fundamental characteristics which segregate cancer stem cells from other stem cells are ability to initiate and regenerate the tumor, representing a phenocopy of the original tumor. These cells exhibit *in vivo* self-renewal capability and demonstrate a unique capacity to differentiate into various lineages, allowing them to give rise to a heterogeneous progeny.[22]

In the recent decade, stem cell markers such as aldehyde dehydrogenase 1, CD271, CD24 and CD44 have been used in the identification of OED and oral squamous cell carcinoma.[23]

In the present study, there was a decline in the expression of CD44 in three grades of epithelial dysplasia when

compared to normal mucosa. The correlation between the degree of dysplasia and CD44 v6 down-regulation might reflect the fact of early cellular changes from normal cell-cell and cell-matrix interactions toward the bizarre, pathophysiological heterotypic cell surface adhesion property, which may be contributory for the cells to achieve invasion and early development of malignant tumors in the oral cavity.[24-26]

The mean of CD44 immunopositive cells was more in normal mucosa when compared to mild, moderate and severe epithelial dysplasia. Severe epithelial dysplasia cases showed down-regulated expression of CD44. The correlation between the degree of dysplasia and CD44 down-regulation has been related to proliferation as well as the grade of cellular differentiation implicated in motility and invasion of the lesion.[23,27,28]

CONCLUSION

CD44 mediates the adhesive properties and signals for the orientation of epithelial cells to migrate upward. It regulates the interaction of growth factors and their corresponding receptors. Increased cellular profileration is a result of enchanced binding of the growth factors with their principle receptors, which is correlated to loss of CD44 and its reduced expression. Thus, CD44, a cell membrane marker could help in cell adhesion and cell-cell interactions. It can be used as a prognostic marker for identifying the rate of malignant transformation and by detecting the severity of the disease.

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Conflicts of interest
There are no conflicts of interest.

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