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Role of alteration of cytokeratin 5/6 profile in dysplastic progression of oral mucosa

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Abstract

Introduction: Squamous cell carcinoma (SCC) rep resents the vast majority (90%) of malignant oral neo plasms. Immuno his to chemistry is a technique which is used for identifying antigens by antigen- anti body interactions. Immuno his to chemistry is extensively used to detect cancers. Cytokeratin's expression is a hall mark of tumor progression.[1]

Cytokeratins, are candidates for OSCC diagnostic marker as they are expressed in OSCC than normal mucosa[2]. Among all, CK5/6 is a basal cytokeratin which normally expresses in squamous epithelium and in squamous cell carcinoma with proliferative potential of these cells[5].

Aim: To evaluate alteration of CK 5/6 expression in different grades of oral dysplastic lesions and to find out usefulness of CK 5/6 as an early detector of oral dysplastic lesions before their conversion into OSCC.

Objectives: To study immunohistochemical expression of CK 5/6 in normal and different grades of oral dysplastic lesions.

Materials And Method: Total 50 (tissue blocks) were collected from archives of oral pathology department of Ahmedabad dental collage during periods of 2020-2023. Out of that 5 blocks were of normal tissue and 45 were of different grades of oral dysplastic lesion. Two sections of 3-4 micron in thickness were cut, of which one section was stained with Haematoxylin and Eosin stain for determining the grade of oral dysplastic lesion according to WHO classification 2005, and another section stained with CK-5/6 for immuno his to chemical staining to assess its expression.

Results: 5 cases of normal mucosa, showed negative CK 5/6 expression intensity. In 15 mild samples taken, intensity of CK5/6 expression was negative in 6 cases (40%), mild in 5 cases (33.33%) and moderate in 4 cases (26. 66%). In 15 moderate samples taken, intensity of CK5/6 positive expression was mild in 2 cases (13. 33%), moderate in 8 cases (53.33%) and severe in 5 cases (33. 33%). In 15 severe samples taken, intensity of CK5 /6 positive expression was mild in 0 cases, moderate in 4 cases (26. 66 %) and severe in 11 cases (73. 33%).

Conclusion: Male predominance (64%) was found in sample of oral epithelial dysplasia. Highest CK 5/6 expression was found in age range of 30 - 39 years (36 %). Also, the number (percentage) of CK 5/ 6 positive cells in samples (Quantitative analysis), increased significantly with increased grades of dysplasia.

Keywords: Squamous cell carcinoma, cytokeratine, immuno his to chemistry

Introduction

Squamous cell carcinoma (SCC) represents the vast majority (90 %) of malignant oral neo plasms.[1] Sometimes OSCC arise de novo but 95% of OSCC develop from a pre-cancerous lesions[2]. Studies have shown that oral dys plastic lesions have tendency to become malignant within 3 years if not treated promptly. During the process of malignant trans formation of oral epithetlium, oral Leukoplakia represents one of the first mor pho logically recognizable epithelial alterations. In this regard, oral Leukoplakia is therefore an important lesion for clinical preventive investigation and an improved understanding of molecular changes during the trans formation process.[3] Apart from proper his to logical assessment of dysplastic lesion, its required to identify additional markers which could facilitate identification of high-risk oral lesions before their progression to malignancy.[2]

There is always a subjective difference to differrentiate well, moderate and poorly differrentiated carcinoma hence immuno his to chemical (IHC) marker are used. Immuno his to chemistry is a technique which is used for identifying antigens by antigen- antibody interactions. Immuno his to chemistry is extensively used to detect cancers.[4]

Among different immuno markers being widely used for OSCC, cytokeratins are focused by researchers in tumor characterization and diagnosis. In order to early detect the lesions with malignant potential, its needed to measure the accurate immuno markers which can help in timely diagnosis of carcinogenetic changes in oral lesions[2.] Some keratins are also known to be altered in malignant trans formation of oral epithelium like oral squamous cell carcinoma (OSCC). CK expression is a hallmark of tumor progression.[1.] Cytokeratins, are candidates for OSCC diagnostic marker as they are expressed in OSCC than normal mucosa[2]. Among all, CK5/6 is a basal cytokeratin which normally expresses in squamous epithelium and in squamous cell carcinoma with proliferative potential of these cells[5]. One of the main dilemma in our society is late presentation of OSCC which ultimately ends up with less chances of

success. According to documentations, early oral epithelial dysplastic lesions are curable One of the main dilemma in our society is late presentation of OSCC which ultimately ends up with less chances of success. According to documentations, early oral epithelial dysplastic lesions are curable[2]. This study began with the aim to find out usefulness of CK5\6 as an early detector of oral dysplastic lesions before they transformed into deadly malignancy like OSCC.

Aim

To evaluate alteration of CK 5/6 expression in different grades of oral dysplastic lesions and to find out usefulness of CK 5/6 as an early detector of oral dysplastic lesions before their conversion into OSCC.

Objectives

To determine grade of oral epithelial dysplastic lesion following WHO classification 2005 in H & E-stained sections. To study immuno his to chemical expression of CK 5/6 in normal and different grades of oral dysplastic lesions. To compare the immuno his to chemical expression of CK 5/6 in normal and different grades of oral dysplastic lesions.

Materials and Method

Total 50 (tissue blocks) were collected from archives of oral pathology department of Ahmedabad dental collage during periods of 2020-2023. Out of that 5 blocks were of normal tissue and 45 were of different grades of oral dysplastic lesion. These were again graded as mild, moderate and severe following WHO classification 2005 using architecture and cytology criteria, 15 blocks of mild, 15 blocks of moderate and 15 blocks of severe dysplasia were selected. Two sections of 3-4 micron in thickness were cut, of which one section was stained with Haema toxylin and Eosin stain for determining the grade of oral dysplastic lesion according to WHO classification 2005, and another section stained with CK-5/6 for immuno his to chemical staining to assess its expression.

Interpretation of staining

Presence of brown coloured end product at the site of target antigen was indicative of positive immuno reactivity. The negative control tissue demonstrated absence of staining and graded as positive or negative.

The positive results were assessed further for number of positive cell for CK5/6 staining, which was graded for statistical analyses. The immuno reactivity was considered positive if 10% or more the tumor cells stained, and was graded either as weak 1 (10-25% of positive cells), mild 2 (26-50% of positive cells), moderate 3 (51-75 % of positive cells) or strong 4 (76-100% of positive cells).

$$\text{Percentage of positive cells} = \frac{\text{Number of positive cells in an area X 100}}{\text{Total number of epithelial cells in an area}}$$

Percentage of positive cells

0 - <10% positive cells

1 - 11-25% positive cells

2 - 26-50% positive cells

3 - 51-75% positive cells

4 - 76-100% positive cells

The positive results were also assessed further for intensity of staining for CK5/ 6 staining and nuclear staining was considered positive, which was graded for statistical analyses. In cases with staining heterogeneity, the expression was grouped according to the predominant staining intensity.

Staining intensity was graded as

0 - No color reaction

1 - Mild color reaction

2 - Moderate color reaction

3 - Strong color reaction

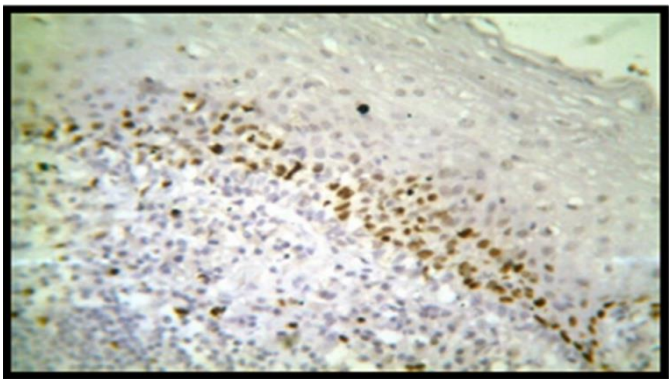

Figure 1: Photo micro graph showing CK5/6 expression in mild oral epithelial dysplasia (IHC, 10X)

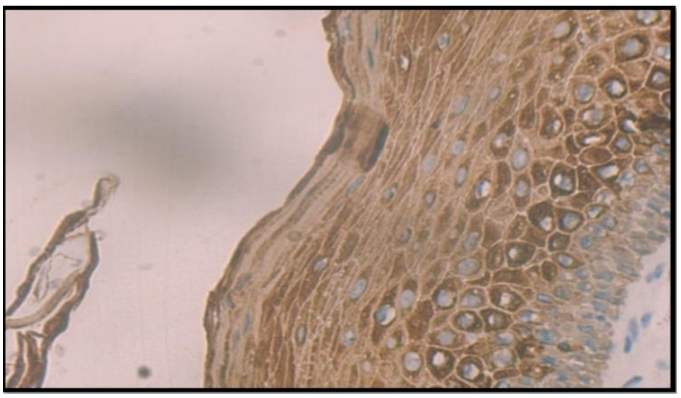

Figure 2: Photomicrograph showing CK5/6 expression in moderate oral epithelial dysplasia (IHC, 40X)

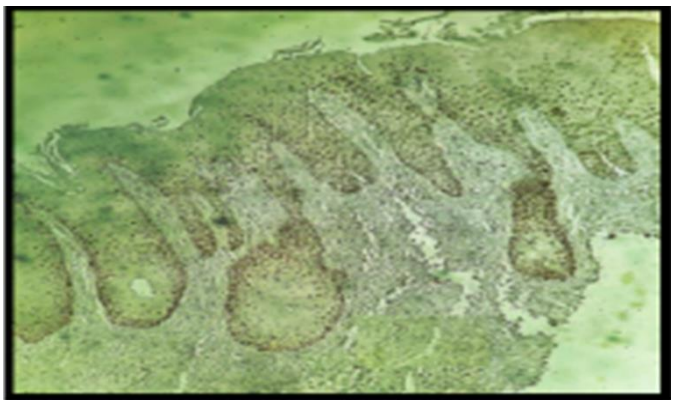

Figure 3: Photomicrograph showing CK 5/6 expression in severe oral epithelial dysplasia (IHC, 10X)

Results

5 cases of normal mucosa, showed negative CK 5/6 expression intensity. In 15 mild samples taken, intensity of CK5/6 expression was negative in 6 cases (40%), mild in 5 cases (33.33%) and moderate in 4 cases (26.66%). In 15 moderate samples taken, intensity of CK5/6 positive expression was mild in 2 cases (13.33%), moderate in 8 cases (53.33%) and severe in 5 cases (33.33%). In 15 severe samples taken, intensity of CK5/6 positive expression was mild in 0 cases, moderate in 4 cases (26.66%) and severe in 11 cases (73.33%). (Table 1).

The positive results were assessed further for number of positive cell for CK5/6 staining, which was graded for statistical analyses. In 5 samples of normal mucosa, only one case (20%) showed weak immuno reactivity for CK 5/6 staining and rest of 4 samples (80%) showed negative immuno reactivity. In 15 samples of mild dysplasia, only one case showed negative immuno reactivity (6. 66%), 10 cases showed weak (66. 66%) and 4 cases (26. 66%) showed mild reactivity. In 15 samples of moderate dysplasia, only 1 case (6.66%) showed weak immuno reactivity, 8 cases (53.33%) showed mild, 5 cases (33. 33%) showed moderate and 1 case (6. 66%) showed strong immuno reactivity. In 15 samples of severe dysplasia, 2 cases (13.33%) showed mild, 5 cases (33.33%) showed moderate and 8 cases (53.33%) showed strong immuno reactivity. (Table 2)

Table 1: Intensity of CK 5/6 positive expression in samples (Qualitative analysis)

Dysplasia	0 (Negative)	1 (Mild)	2 (Moderate)	3(Strong)
Normal (5)	05	00	00	00
Mild (15)	06 (40%)	05 (33.33%)	04 (26.66%)	00
Moderate (15)	00	02 (13.33%)	08 (53.33%)	05 (33.33%)
Severe (15)	00	00	04 (26.66%)	11 (73.33%)

Chi Square Value: 47.863 P Value: <0.0001

Table 2: Number (Percentage) of CK 5/6 positive cells in samples (Quantitative analysis)

One way ANOVA								
Descriptives								
Percentage of CK 5/6 in +ve cells								
	N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
NORMAL	5	8.40	8.79	3.93	-2.52	19.32	3	24
MILD	15	19.67	10.57	2.73	13.81	25.52	9	46
MODERATE	15	50.53	17.16	4.43	41.03	60.04	23	80
SEVERE	15	74.20	20.70	5.35	62.74	85.66	29	98
Total	50	44.16	29.12	4.12	35.89	52.43	3	98

Chi Square Value:72.296 P Value:<0.0001

Table 3: Statistical analysis of number of C/K positive cell in different grades of oral Epithelial Dysplasia.

	0	1	2	3	4
	< 10 %	11-25 %	26-50 %	51-75 %	76-100 %
Normal	4 (80%)	1 (20%)	-	-	-
Mild Dysplasia	1 (6.66%)	10 (66.66%)	4 (26.66%)	-	-
Moderate Dysplasia	-	1 (6.66%)	8 (53.33%)	5 (33.33%)	1 (6.66%)
Severe Dysplasia	-	-	2 (13.33%)	5 (33.33%)	8 (53.33%)

Discussion

The pathogenesis of squamous cell carcinomas is poorly understood. A variety of cell biological markers mainly involved in cell proliferation and apoptosis have been described.

An improved understanding of the biology of squamous cell carcinoma (SCC) of the oral cavity and its proposed precursors in some instances such as oral Leukoplakia with and without dysplasia could potentially identify prognostic factors allowing tailoring of preventive and surgical strategies.

Cytokeratins are the leading bare the leading bio markers in diagnostic pathology[6]. Cytokeratin pair seems to play an important role in the pathogenesis and progression of oral squamous cell carcinoma. cytokeratins 5 and 6 are often jointly examined by immuno his to chemistry because common antibodies recognize both cytokeratins 5 and 6 and the use of these bispecific antibodies has clinical utility. Cytokeratin 5/6 (CK5/ CK6) antibodies are for example applied to identify basal cells or myoepithelial cells for ruling out invasive breast and prostate cancer, to detect squamous cell origin in poorly differentiated carcinomas[7]. In our study for 45 dysplastic samples, 29 (64.44%) were from males and 16 (35.55%) were from females. Most patients were in the age range of 40-60 years. It showed a trend towards younger patients getting affected by oral cancer, which was same as Batool S et al in 2020[2]. In present study, 5 cases of

normal mucosa, showed negative CK 5/6 expression intensity. Our study showed positive expression of CK5\6 in 100% of dysplastic samples. These findings were in consistent with the results found by Fillies T et al in 2007 showed a strong expression of CK5 \6 in all of the oral pre-cancerous lesions with significant association[3]. In our study 10% of the samples of normal oral mucosa were negative for CK5\6 which was in similarity with the studies done by Batool S et al in 2020, showing complete absence or weak staining of CK5\6 in non-keratinized oral mucosa.[2] Our results were in contrast with the study done by Shahabinejad M et al in 2021 who found no correlation of cytokeratin expression with demographic and his to pathological characteristics, including grade and stage of the tumors. They found no significant difference between cytokeratin expression and age or gender. However, they also found significant correlation of cyrtokeratin expression with higher grade tumors.[8]

One interesting finding of our study was the sequential increase in the staining expression of CK5\6 from normal oral mucosa to various grades of dysplastic lesions. Apart from that, studies done by Ranganathan et al in 2006 and Vaidya et al in 2000 conflicted our results that under expression of CK5 alone was related with indication of dysplastic lesions of oral cavity. Although, study conducted by Fillies T showed no correlation between malingnant trans formation and expression of CK 5/6.[9]

The positive results were assessed further for number of positive cell for CK5/6 staining, which was graded for statistical analyses in the present study. Our study was in consistent with study done by Hashmi et al and Volkel C et al who found significant association of CK 5/6 expression with tumor grade and muscularispropria invasion. Study done by Bhalla et al in 2010 showed significant difference in staining characteristics of CK5/ 6 in benign and malignant tumors of breast.[10]

Chu PG at al in 2002 studied immunoreactivity of CK5/ 6 in epithelial tumors from various organs. In contrast to our study, they found no difference in staining in squamous cell and basal cell carcinomas showing different grades of differentiation. However, they found difference in CK 5/6 staining pattern.[11]

Conclusion

Male predominance (64%) was found in sample of oral epithelial dysplasia. Highest CK 5/6 expression was found in age range of 30-39 years (36%). Also, the number (percentage) of CK 5/6 positive cells in samples (Quantitative analysis), increased significantly with increased grades of dysplasia. ($p < 0.0001$) There was significant increase in intensity of CK 5/6 staining (qualitative analysis) with increased grades of oral epithelial dysplasia. ($p < 0.0001$) Strong immuno reactivity was observed in severe dysplasia as compared to mild and moderate dysplasia.CK5\6 is being currently in use for the diagnostic purposes of oral squamous cell carcinoma due to their active role in carcinogenesis but we observed highly significant and sequential amplifyication of CK5\ 6 in transition from normal oral mucosa to early grades of oral epithelial dysplastic lesions.

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