ORIGINAL ARTICLE

ROLE OF ALTERATION OF CK5\6 PROFILE IN DYSPLASTIC PROGRESSION OF ORAL MUCOSA IN TOBACCO USERS

Sarwat Batool, Amin Fahim*, Aneela Qureshi, Humaira Jabeen, Syed Naqeeb Ali, Mohammad Yousuf Khoso

Department of Pathology, Al-Tibri Medical College, Isra University Karachi Campus, *Department of Pathology, Isra University Hyderabad-Pakistan

Background: To determine staining expression of CK5\6 in healthy oral mucosa and various grades of oral dysplasia and to find out possible association of CK5\6 expression in dysplastic transformation of clinically normal oral mucosa to various grades of oral dysplasia. **Methods:** This cross-sectional descriptive study was done at Al-Tibri Medical College and Hospital and Dow University of Health Sciences, Karachi from March 2018 to November 2018. It included 120 diagnosed paraffin embedded tissue samples of normal oral mucosa and various grades of oral epithelial dysplastic lesions. Patient's data was reviewed for age, gender and tobacco habits. For immunohistochemistry CK5\6 staining was performed on all the samples. Immunohistochemical evaluation was done by observing the staining expression of CK5\6 on various oral dysplastic samples on the basis of staining intensity. The compiled data was statistically analyzed by using Chi-square. *p*-values of <0.05 were considered to be significant. **Results:** All of the 60/60 (100%) oral dysplastic cases were moderately to strongly positive for CK5\6. Gradual increase in staining intensity for CK5\6 was observed with increasing grades of dysplasia. We found highly significant association of CK5\6 immunopositivity in transformation of normal mucosa to various grades oral dysplastic lesions. **Conclusion:** CK5\6 can be used as reliable adjuvant marker for the early dysplastic transformation of oral mucosain tobacco users, before it progresses to oral squamous cell carcinoma (OSCC).

Keywords: Oral dysplastic lesions; CK5\6; Oral squamous cell carcinoma

Citation: Batool S, Fahim A, Qureshi A, Jabeen H, Ali SN, Khoso MY. Role of alteration of CK5\6 profile in dysplastic progression of oral mucosa in tobacco users. J Ayub Med Coll Abbottabad 2020;32(4):527–30.

INTRODUCTION

Oral cavity is vulnerable to any endogenous or exogenous pathological changes in body.[1] Cytokeratin's are intracytoplasmic structural proteins which are engaged in stabilizing the shape of cell and nucleus and resist unwanted forces acting upon cell.[2] These are found in almost all epithelial cells of body and consists of about 30 different proteins with complex high molecular weight (HMW) with 40–50 kd or low molecular weight (LMW) proteins ranging from 50–60 kd.[3] These filaments are known to be involved in various intracellular pathways as well as cell migration processes in normal oral epithelial cells. Apart from this some keratins are also known to be altered in malignant transformation of oral epithelium like oral squamous cell carcinoma (OSCC).[4] Sometimes OSCC arise de novo but 95% of OSCC develop from a precancerous lesions.[5] Studies have shown that oral dysplastic lesions have tendency to become malignant within 3 years if not treated promptly.[6] The prognostic significance of CK5\6 has not been proved for oral dysplastic lesions but their altered presence in OSCC have increased the awareness for cytokeratin disturbances in oral carcinogenesis.[7] Tobacco is one of the most important risk factor for OSCC. Extent of OSCC also depends upon type of tobacco i.e. smoked or chewable, duration and frequency of tobacco usage.[8] The tobacco results in epigenetic changes which interfere with activation and degradation of carcinogens.[9]

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.[10] Therefore 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 especially in those individuals with risk factors like tobacco use.

MATERIAL AND METHODS

This cross-sectional descriptive study was carried out in Al-Tibri Medical College and Hospital (ATMCH) and Dow University of Health Sciences (DUHS) from March 2018 to November 2018. Sample size was determined by using Cochrane's formula at confidence level (Z1-a/2) of 95%. Probability was assumed at 4% and margin of error was considered at 5%.[11] A total of 120 histologically diagnosed, formalin-fixed, paraffin embedded tissue samples were collected. These included 60 samples from healthy oral mucosa and 60 samples from of oral dysplastic lesions. Reconfirmation of

histopathological grading of samples was done by experienced pathologists. All of the blocks from dysplastic lesions were categorized into different grades of dysplasia based on WHO criteria for oral dysplasia 2005 into mild dysplasia (20 cases), moderate dysplasia (21) and severe dysplasia (19).[12] OSCC samples was taken as positive control and samples with exclusion of primary antibody was taken as negative control.

Patient's details about age, gender, tobacco habits and initial clinical diagnosis were recorded from their medical records. From each of the representative tumor tissue block, 2 sections of 5mm thickness were cut and stained respectively by hematoxylin and eosin stain for histopathological grading. Another section was utilized for immunohistochemical staining with CK5\6. Sections were processed for immune-histo-chemistry like dewaxing, clearing in xylene and re-hydratingin descending grades of alcohol. Antigen retrieval was carried out in a pressure cooker using citrate buffer for 2–5 min. Then slides were allowed to cool down at room temperature. The sections were incubated in 3% hydrogen peroxide solution for 15 min to block endogenous peroxidase activity and were incubated for 1 h with primary monoclonal anti CK5\6 antibody (clone D5\16B4, dilution 1:25 Dakocytomotion). Sections then were incubated for 45 min in secondary antibody. Finally, for antigen visualization, the slides were incubated for 10 min with chromogen and counterstained by Harris hematoxylin. All the above-mentioned steps were carried out at room temperature and after each step the sections were washed with buffer saline. Immunohistochemical evaluation was done by qualitative method for grading of staining intensity for positive cells after processing of samples with CK5\6. These included the proportion of area stained with light microscope under x40 and intensity of staining under x100. Any homogenous brown cytoplasmic and\or membranous staining at >10% area was considered as positive and those cases were considered as negative in which only counterstain was visible.[13] The compiled clinical, histopathological and immunohistochemical data was statistically analyzed using the SPSS 20.0, IBM Corporation, New York, U.S. Chi-square test was used to determine the statistical significance between the different parameters. Correlation between CK5\6 intensity and grade of dysplasia was determined by Pearson's correlation. *p*-values of <0.05 were considered significant.

RESULTS

Frequencies and percentages of the collected demographical and clinicopathological data are shown in Table-1, which shows male gender (67%), patients with age <50 years (79%) and mean age of 45±5 years, regular tobacco consumption (86%) and buccal mucosa as the most commonly seen variables (73%).

According to findings in Table-II, CK5\6 expression in normal oral mucosa was mild and sparse but all the samples of oral dysplasia were moderately to strongly positive for CK5\6 strong expression. Significance of association of CK5\6 expression among study groups revealed that CK5\6 is significantly associated with increasing degree of oral dysplasia. We further found significant correlation of increasing CK5\6 intensity with increasing grades of dysplasia (*p*-value=0.77). Representative cases of CK5\6 immunostaining among various study groups are shown in figure-1.

Table-1: **Overall demographical and clicopathological data with frequencies and percentages**

Variables	Total (n=120)
Gender	
Male	80 (67%)
Female	40 (33%)
Age	
<50 years	95 (79%)
>50 years	25 (21%)
Site	
Buccal mucosa	88 (73%)
Gingival mucosa	15 (13%)
Alveolar mucosa	16 (13%)
Tongue	1 (1%)
Tobacco	
Regular users	103 (86%)
Occasional users	17 (14%)
Type of mucosa	
Normal mucosa	60 (50%)
Mild dysplasia	20 (33%)
Moderate dysplasia	19 (35%)
Severe dysplasia	21 (32%)

Table-2: **Association of CK5\6 expression on various study groups (n=120)**

Groups	CK5\6 expression				*p*-value
	Negative	Mild staining	Moderate staining	Strong staining	
Normal oral mucosa (n=60)	12 (20%)	48 (80%)	0	0	
Mild dysplasia (n=20)	0	0	5 (25%)	15 (75%)	
Moderate dysplasia (n=21)	0	0	8 (38%)	13 (62%)	
Severe dysplasia (n=19)	0	0	6 (32%)	13 (68%)	0.002

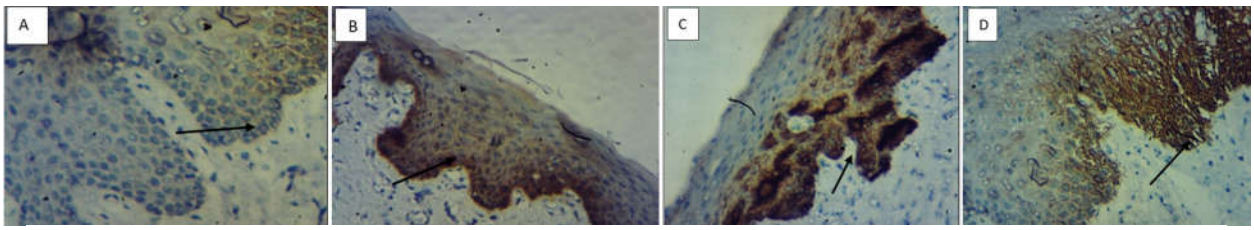

Figure-1: Representative photomicrographs of CK5\6 expression on normal oral mucosa and various oral dysplastic lesions

A. An arrow shows faint and sparse brown membranous staining limited to basal layers of normal oral mucosa with CK5\6 x100. **B.** An arrow shows presence of strong brown cytoplasmic staining of CK5\6 on mild dysplastic oral epithelium at x100. **C.** An arrow shows presence of strong brown cytoplasmic and membranous staining of CK5\6 on moderate dysplastic oral epithelium at x100. **D.** An arrow shows presence of intense brown cytoplasmic and membranous staining of CK5\6 on severe dysplastic oral epithelium at x100.

DISCUSSION

Unfortunately despite of advanced diagnostic modalities, OSCC is still one of a major threat to survival rate of population of Pakistan, i.e., less than 50%.[14] Therefore apart from proper histological assessment of dysplastic lesion, its required to identify additional markers which could facilitate identification of high risk oral lesions before their progression to malignancy.[15] Among different immunomarkers being widely used for OSCC, cytokeratins are focused by researchers in tumor characterization and diagnosis.[16] In order to early detect the lesions with malignant potential, its needed to measure the accurate immunomarkers which can help in timely diagnosis of carcinogenetic changes in oral lesions.[17]

In our study 20% of the samples of normal oral mucosa were negative for CK5\6 which was in similarity with the studies done by Mackenzie I in 1993, Jiang Q in 2014 and Kaufmann O in 2002 showing complete absence or weak staining of CK5\6 in non-keratinized oral mucosa.[18–20] This could be explained by the evidence given by Chattarjee S in 2012, that CK 5 alone showed positive expression in basal layers of non-keratinized epithelium but its pair with CK 6/5 showed absence of staining or weak staining.[21] Another studies also supported this fact and stated that CK 5/6 was a marker for proliferation and hence showed staining only in proliferating epithelium and not in normal non proliferating epithelium.[18,21]

As mentioned earlier that cytokeratin might be present in the suprabasal layers of keratinocytes of healthy keratinized and few non keratinized oral epithelium,[7] therefore it could be the reason for Ck5\6 expression in 80% samples of normal mucosal samples with mild staining expression as few samples were obtained from tissue blocks of tongue and masticatory gingiva which had keratinized stratified squamous epithelium. Similar results were given by Kasai *et al* in 2016, who showed expression of CK1 to CK20 in tissues taken from buccal, labial and gingival areas and showed strong expression of Ck5\6 in middle and upper regions of keratinized oral epithelium.[22]

Apart from strong expression of Ck5\6 in normal oral mucosa, we were unable to find any study which could show grading of staining expression of Ck5\6 in normal mucosal cells therefore it was not possible to compare the staining grades found in our results.

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 precancerous lesions with significant association.[4] 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 Vaida *et al* in 2014 conflicted our results that under expression of CK5 alone was related with indication of dysplastic lesions of oral cavity.[23,24] Noteworthy we were unable to compare and conflict our result for CK5\6 paired expression in oral dysplastic lesions as it had been discussed earlier that there was very less data in that context and more of the studies for oral precancerous lesions were being done on focusing unpaired deregulation of CK5 or CK6.

CONCLUSION

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 amplification of CK5\6 in transition from normal oral mucosa to early grades of oral epithelial dysplastic lesions. In the view of our results CK5\6 could be a possible diagnostic tool for early oral dysplastic lesions as well. Further cohort study designs are required for better understanding the potential of paired deregulation of CK5\6 marker as early indicator of oral dysplasia.

Source of Funding: This project was completed by M.Phil funding, given by Al-Tibri Medical College, Isra University, Karachi Campus.

AUTHORS' CONTRIBUTION

SB: Study concept and design, critical revision. AF: Drafting of manuscript, acquisition of data. AQ: Critical revision, analysis and interpretation of data. HJ: Literature search, write-up. SNA: Data analysis, proof reading, final approval. MYK: Write-up, data collection.

REFERENCES

1. Khan SZ, Farooq A, Masood M, Shahid AB, Irfan UK, Nisar H, *et al*. Smokeless tobacco use and risk of oral cavity cancer. Turk J Med Sci 2020;50(2):291–97.
2. White JK, Gerdin AK, Karp NA, Ryder E, Buljan M, Bussell JN, *et al*. Genome-wide Generation and Systematic Phenotyping of Knockout Mice Reveals New Roles for Many Genes. Cell 2013;154(2):452–64.
3. Miller RD. Cytokeratin 5\6. Propath. [Internet]. 2000. [cited 2019 May]. Available from: http://propath.org/companies/press-clippings/26-newsletters/305-cytokeratin-5-6-december-2000
4. Fillies T, Jogschies M, Kleinheinz J, Brandt B, Joos U, Buerger H. Cytokeratin alteration in oral leukoplakia and oral squamous cell carcinoma. Oncol Rep 2007;18(3):639–43.
5. Parlatescu I, Gheorghe C, Coculescu E, Tovaru S. Oral Leukoplakia - an Update. Maedica (Buhar) 2014;9(1):88–93.
6. Premalignant lesions. The oral cancer foundation; [Internet]. 2018. [cited 2019 May]. Available from: https://oralcancerfoundation.org/cdc/premalignant-lesions/
7. Frohwitter G, Guerger H, Van Diest PJ, Korsching E, Kleinheinz J, Fillies T. Cytokeratin and protein expression patterns in squamous cell carcinoma of the oral cavity provide evidence for two distinct pathogenetic pathways. Oncol Lett 2016;12(1):107–13.
8. Beynon RA, Lang S, Schimansky S, Chrisopher MP, Weylen A, Thomas SJ, et al. Tobacco smoking and alcohol drinking at diagnosis of head and neck cancer and all-cause mortality: Results from head and neck 5000, a prospective observational cohort of people with head and neck cancer. Int J Cancer 2018;143(2):1–14.
9. Akram S, Mirza T, Aamir Mirza M, Qureshi M. Emerging Patterns in Clinico-pathological spectrum of Oral Cancers. Pak J Med Sci 2013;29(3):783–7.
10. Devi M, Vijayalakshmi D, Dhivya K, Vezhavendhan N. A study on awareness of early detection and screening of potentially malignant oral disorders and oral cancer. J NTR Univ Health Sci 2017;6(1):35–8.
11. Charan J, Biswas T. How to calculate sample size for different study designs in medical research. Indian J Psychol Med 2013;35:121–6.
12. Mortazavi H, Baharvand M, Mehdipour M. Oral Potentially Malignant Disorders: An Overview of More than 20 Entities. J Dent Res Dent Clin Dent Prospects 2014;8(1):6–14.
13. Kitamura R, Toyoshima T, Tanaka H, Kawano S, Kiyosue T, Matsubara R, *et al*. Association of cytokeratin 17 expression with differentiation in oral squamous cell carcinoma. J Cancer Res Clin Oncol 2012;138(8):1299–310.
14. Sarode SC, Sarode GS, Karmarkar S, Tupkari JV. A new classification of potentially malignant disorders of the oral cavity. Oral Oncol 2011;47(9)920–1.
15. Ruiter DJ, Fleuren GJ, Warnaar SO. Application of Monoclonal Antibodies in Tumor Pathology. Martinus Nijhoff Publishers. BOSTON, 2012; p.64.
16. Pernick N. Cytokeratins (CK) - general. Pathology Outlines. [Internet]. 2013. Cited 2019 May]. Available from: http://www.pathologyoutlines.com/topic/stainsckgeneral.html
17. Patil A, Patil K, Tupsakhare S, Gabhane M, Sonune S, Kandalgaonkar S. Evaluation of Podoplanin in Oral Leukoplakia and Oral Squamous Cell Carcinoma. Scientifica 2015;2015:1–6.
18. Mackenzie I, Gao Z. Patterns of cytokeratin expression in the epithelia of inflamed human gingiva and periodontal pockets. J Periodontal Res 1993;28(1):49–59.
19. Jiang Q, Yu Y, Ruan H, Luo Y, Guo X. Morphological and functional characteristics of human gingival epithelium. BMC Oral Health 2014;14(1):30.
20. Kaufmann O, Fietze E, Mengs J, Dietel M. Value of p63 and cytokeratin 5\6 marker in immunohistochemistry of differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2002;116(6):823–30.
21. Chatterjee S. Cytokeratins in health and diseases. Oral Maxillofac Pathol J 2012:3(1);198–202.
22. Kasai Y, Sugiyama H, Takagi R, Kondo M, Owaki T, Namiki H, *et al*. Brush biopsy of human oral mucosal epithelial cells as a quality control of the cell source for fabrication of transplantable epithelial cell sheets for regenerative medicine. Regen Ther 2016;4:71–7.
23. Ranganathan K, Kavitha R, Sawant SS, Vaidya MM. Cytokeratin expression in oral submucous fibrosis Animmunohistochemical study. J Oral Pathol Med 2006;35(1):25–32.
24. Sawant S, Vaidya M, Chaukar D, Gangadaran P, Singh AK, Rajadhyax S, *et al*. Clinicopathological features and prognostic implications of loss of K5 and gain of K1, K8 and K18 in oral potentially malignant lesions and squamous cell carcinomas: An immunohistochemical analysis. Edorium J Tumor Biol 2014;1:1–22.

Submitted: June 10, 2019	*Revised: --*	*Accepted: January 12, 2020*

Address for Correspondence:

Dr. Amin Fahim, Department of Pathology, Isra University, Hyderabad-Pakistan
Cell: +92 331 350 4341
Email: draminfahim@gmail.com