

Aberrant expression of CK8 in leukoplakia and oral squamous cell carcinoma: an immunohistochemistry study

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Abstract

Introduction: In India, oral cavity cancer contributes to significant morbidity and mortality. Since only few of premalignant lesions turn into cancer and routine histopathology has limited prognostic value, therefore we need to devise methods to ascertain premalignant lesions with high potential for conversion to malignancy, appropriate management and early diagnosis of cancers. Cytokeratin K8 (CK8) may be used as surrogate markers in oral premalignant lesion and cancers, so we conducted the present study with an aim to evaluate & compare expression of CK8 in normal oral mucosa, leukoplakia as well as Oral squamous cell carcinoma (OSCC). **Material and Methods:** The present study included 107 pathological specimens of histologically proven cases of OSCC (n=19), leukoplakia (n=77) and benign lesions (n=10). All the cases were subjected to immunohistochemistry to check the expression of CK8 antibodies. Correlation was evaluated statistically by Chi-square test. **Result:** Increased expression of CK8 was seen in OSCC and leukoplakia with dysplasia. No immunoreactivity was seen in benign oral mucosa. Expression of CK8 was significantly correlated with dysplasia and cancer. **Conclusion:** CK8 can be used as marker of sequential premalignant changes in oral cancers.

Keyword: CK8, Leukoplakia, Oral squamous cell carcinoma

Introduction

Oral cancer is the sixth largest group of malignancies in the world [1]. Squamous cell carcinoma, accounts for 95% of all oral malignancies [2]. Tobacco coupled with alcohol consumption, low socioeconomic status and poor oral hygiene are acknowledged risk factors for oral cancer [3]. OSCC may be preceded by premalignant lesions. Leukoplakia with dysplasia and oral submucous fibrosis are the most common premalignant lesions in oral cavity [4]. The risk of malignant transformation of leukoplakia varies from 0.5 to 20% [5]. Histopathological assessment based on dysplasia as predictive marker for malignant transformation of premalignant lesions is subjective and less sensitive [6,7].

Despite the advances in surgery and radiotherapy, the prognosis of OSCC has remained poor, with survival rate of around 50% [8]. Considering all these facts, it is important to devise methods as an adjunct to histopathology to ascertain premalignant lesions with high potential for conversion to malignancy and diagnosis of OSCC. Cytokeratin (CK) is an intermediate filament found mainly in mammalian epithelial cells and form part of cytoskeleton. These CKs are classified into two groups: type I (acidic, CK9-20) and type II (neutral-basic, CK1-8) [9].

Alterations in CK8 expression have been reported in the OSCCs and its precursor lesions [10]. Hence in this study our aim was to analyze and compare expression of CK8 in normal, hyperplastic, dysplastic oral mucosa and OSCC by immunohistochemistry(IHC).

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Material and Methods

The present study was a retrospective study conducted in pathology department, MLB Medical College, Jhansi between years 2013 to 2014. The tissue material for the study was obtained from various out patients and inpatients admitted in ENT department.

This study included 107 total cases: 19 cases of Oral Squamous Cell Carcinoma, 77 leukoplakia and 10 Benign and Inflammatory oral lesions.

Only biopsy proven cases were included. Inadequate biopsies were excluded from the study. Histological examination of biopsies for grading of lesion was done and lesions were divided into benign, hyperplastic, dysplastic and squamous cell carcinoma. Cases of dysplasia were further categorized into mild, moderate and severe, as per WHO consensus.

Result

CK8 was expressed in 15.5% (7/45) cases of hyperplastic epithelium, 62.5 % (10/16) cases of mild dysplasia, 63.63 % (7/11) cases of moderate dysplasia, 100 % (5/5) cases of severe dysplasia and 63.15% (12/19) cases of OSCC. (Table 1). P value of .02 was recorded.

All the cases were subjected to immunohistochemistry to check the expression of antibodies K8 on formalin fixed, paraffin embedded tissue sections by using ready to use C51 monoclonal primary antibody, supplied by Biogenex. Negative controls were conducted in the absence of primary antibodies for every detection system. Breast Carcinoma was taken as positive control

IHC Interpretation: Cytoplasmic staining was taken as positive in epithelium. The percentage was classified in two groups (0: no Expression, 1: $\geq 1\%$ positive expression). Sample evaluation was performed by two pathologists.

Statistical Analysis: Chi-square test was performed to analyze positivity in normal, hyperplasia, dysplasia and OSCC for CK8. Differences with a probability value of <0.05 were considered statistically significant.

Table-1: Presence of CK8 in epithelium of different group.

Histopathological type	CK8 expression			
	-		+	
	N	%	n	%
Normal mucosa (n=10)	10	100	0	0
Hyperplasia (n=45)	38	84.5	7	15.5
Mild Dysplsia (n=16)	6	37.5	10	62.5
Moderate Dysplasia(n=11)	4	36.37	7	63.63
Severe Dysplasia(n=5)	0	0	5	100
OSCC(n=19)	7	36.85	12	63.15

Table-2: Table of Significance (Chi-Square Test)

Histopathological type	TOTAL	Positive ck8 expression	Row Totals
Hyperplasia	45 (36.44) [2.01]	7 (15.56) [4.71]	52
Mild Dysplsia	16 (18.22) [0.27]	10 (7.78) [0.63]	26
Moderate Dysplasia	11 (12.61) [0.21]	7 (5.39) [0.48]	18
Severe Dysplasia	5 (7.01) [0.58]	5 (2.99) [1.35]	10
OSCC	19 (21.72) [0.34]	12 (9.28) [0.80]	31
Column Totals	96	41	137 (Grand Total)

Figure 1,2 and 3 shows staining results of hematoxylin & eosin (H&E) and immunohistochemical staining of hyperplasia, dysplasia and OSCC respectively.

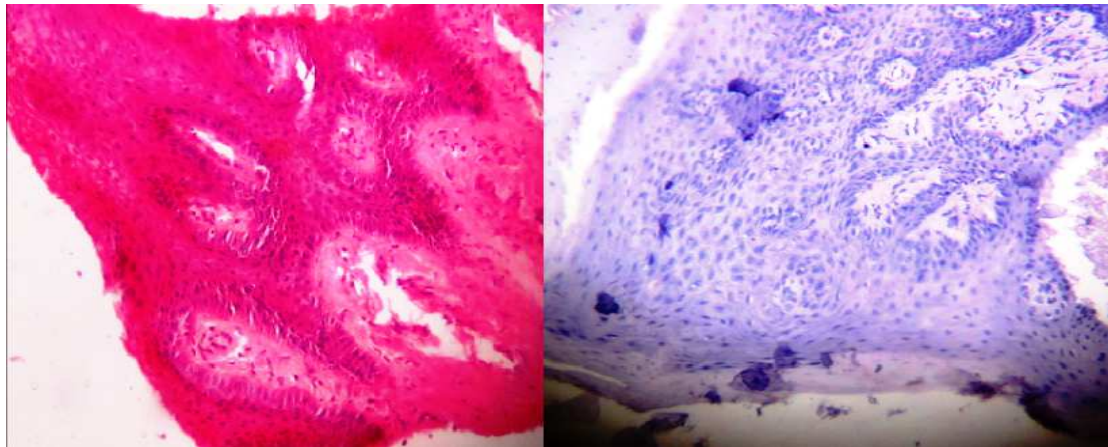


Figure-1: Photomicrograph showing hyperplastic epithelium. Corresponding section showing no immunostaining with anti CK8

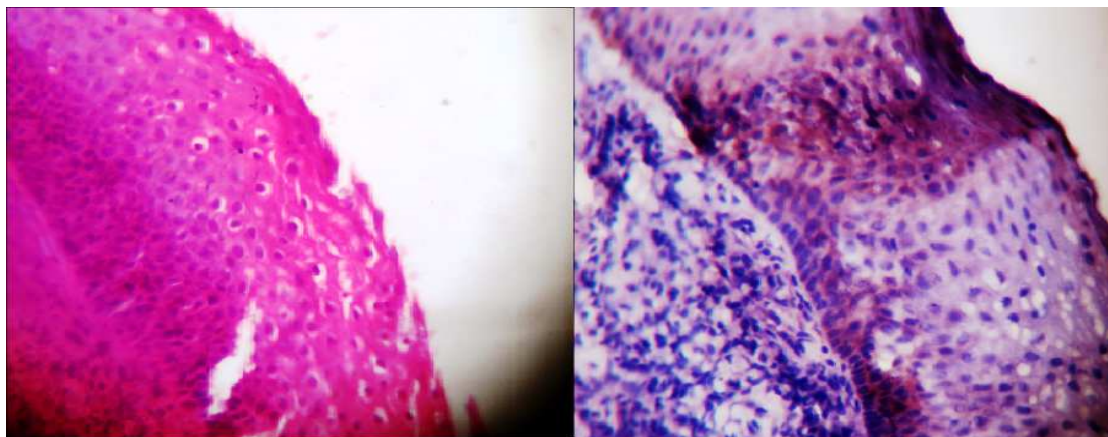


Figure-2: Photomicrograph showing dysplasia characterised by basal cell crowding, increased mitosis & N/C ratio (H&E, x200). Corresponding section showing expression of CK8 in basal & suprabasal layer

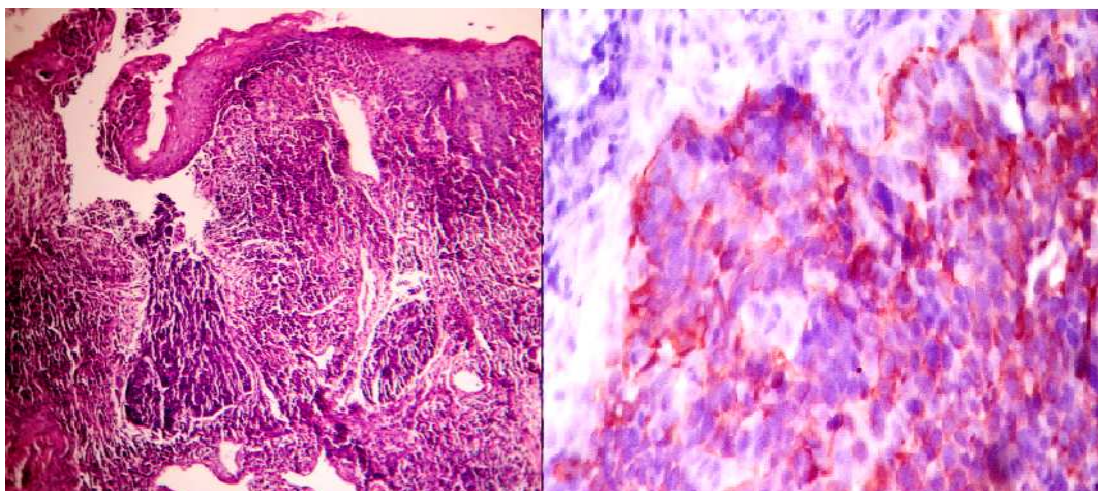


Figure-3: Photomicrograph showing infiltrating island of SCC with limited keratin formation (H&E, x100). Corresponding section showing moderate immunostaining with anti CK8

Aberrant CK8 expression was detected in cases of dysplasia and OSCC but no immunoreactivity was seen in normal oral mucosa. Expression of CK8 was seen in basal and suprabasal layer of epidermis in dysplasia.

Discussion

Oral cancer accounts for 2%–4% of all cancer cases worldwide. It is the 3rd most common type of cancer which accounts for about 30% of all cancers in India [11]. Around 95% of these cancers are squamous cell carcinomas. The most affected sites are ventral surface of the tongue, floor of the mouth, lower lip, soft

palate and gingiva. Tobacco and alcohol are the two most important risk factors. The use of tobacco in form of betel quid is thought to be major cause of OSCC in India. HPV types 16 and 18 have been also implicated in oral cancer [12]. Other risk factors are dietary deficiencies, poor oral hygiene and chronic candidiasis. Despite the advances in therapeutic and diagnostic modalities, the prognosis of OSCC has remained poor, with 5-year survival rate of around 50%. OSCC is often preceded by premalignant lesions.

Identifying a premalignant lesion or carcinoma in early stages will prevent development of malignancy and will provide better survival rates with nominal disfigurement and functional disability [13].

Leukoplakia with dysplasia and oral submucous fibrosis are the most common premalignant lesions of oral cavity. According to WHO, leukoplakia is defined as “a white patch or plaque which cannot be characterized clinically or pathologically as any other disease”. Leukoplakia is a clinical term and should not be used as a histopathological diagnosis. Leukoplakia is mainly a hyperkeratotic response to an irritant and generally asymptomatic, but around 20% of lesions show evidence of dysplasia or carcinoma at first clinical examination. [14].

Majority of oral epithelium is nonkeratinized. However it can undergo abnormal keratinisation in leukoplakia and OSCC, which is accompanied by changes in expression of cytokeratins [15]. Alterations in CK8 expression have been reported in the OSCCs and its precursor lesions.

CK8 is a structural protein and is expressed in normal glandular epithelium, transitional cell epithelium, and hepatocyte, but not in squamous stratified epithelium [16]. CK8 expression may help in differentiating dysplastic lesions, carcinomas in situ, and small carcinomas from normal tissue and hyperplastic lesions within oral leukoplakia [17].

In this study, no expression of CK8 was seen in normal oral mucosa, 15.5% of hyperplastic lesion and 68.75% of dysplastic lesion. This was in accordance with Ogden et al, who demonstrated upregulated expression of CK8 in dysplastic premalignant head and neck lesions and that CAM 5.2 antibody can distinguish between hyperplastic and dysplastic head and neck lesions [18].

In our study CK8 expression was present in 37.66% (29/77) cases of leukoplakia, which is in contrast to 50% (31/62) cases of leukoplakia reported by Sharada Sawant et al [19]. In this study CK8 expression was present in 68.75% (22/32) cases of leukoplakia with dysplasia, which is in contrast to 20-25% cases reported by Vigneswaran et al [20].

In the present study CK8 was expressed in 15.5% (7/45) cases of hyperplastic epithelium, 62.5% (10/16) cases of mild dysplasia, 63.63% (7/11) cases of moderate dysplasia and 100% (5/5) cases of severe dysplasia.

This was in accordance with Sawant et al, who reported CK8 expression in 16% (5/31) cases of hyperplastic epithelium, 64% (7/11) cases of mild dysplasia, 71% (5/7) cases of moderate dysplasia, 100%(3/3) cases of severe dysplasia [21]. In our study CK8 was expressed in 63.15% (12/19) cases of OSCC, which is in contrast to 30% of OSCC cases reported by Nanda et al [22].

In vitro studies have shown that transfection of CK8/18 in non-malignant buccal mucosa cells leads to significant phenotypic changes including increased cellular motility, which might give hints for increased tumor aggressiveness and bad prognosis [23].

Current treatment modalities including surgery, chemotherapy and radiotherapy are aggressive and cause significant morbidity with severe toxicity and functional impairment such as swallowing and speech difficulties. Biomarkers linked with malignant transformation may provide a nonsurgical therapeutic aid as targeted molecular therapy and can complement other existing cancer therapies. Molecules associated with proliferation and differentiation of OSCC like epidermal growth factor receptor and progesterone receptor are being studied [24]. Further studies regarding role of CK8 in malignant transformation of OSCC may help in development of newer targeted therapy.

Conclusion

The present study, demonstrated aberrant expression of CK8 in leukoplakia and OSCC. CK8 expression was correlated with differentiation and thus can be used as a sequential marker of premalignant changes and remove the inter observer bias in assessment of dysplasia.

Further studies are warranted in order to prove the usefulness of CK8 as surrogate marker in diagnosis of potentially malignant oral lesions and its prognostic value in oral cancers.

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