RESEARCH ARTICLE

Differential Expression of Cytokeratin 13 in Non-Neoplastic, Dysplastic and Neoplastic Oral Mucosa in a High Risk Pakistani Population

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Abstract

__Background__: Gradual loss of cytokeratin 13 (CK13) may be linked with the severity of dysplastic changes and transformation to malignancy. In this study we assessed the differential expression of CK13 in normal, hyperplastic, dysplastic and cancerous oral mucosa. __Materials and Methods__: A total of 93 oral biopsies were collected during the 2011-2014 period. The biopsies were characterized as normal (19), hyperplastic (21), severely dysplastic/carcinoma in situ (16) and invasive oral squamous cell carcinoma (OSCC) (37) after morphological assessment. Formalin fixed paraffin embedded sections were stained with a monoclonal antibody against CK13 using the Envision technique. Immunohistochemically stained slides were then analyzed for CK13 expression. __Results__: CK13 was consistently and diffusely expressed in all normal and hyperplastic tissue biopsies from oral mucosa. Severely dysplastic/carcinoma in situ biopsies showed complete loss in 50% of cases, while in the remaining 50% expression was very focal and weak. OSCC cases showed complete or near complete loss of CK13 in all cases. Few cases showed weak expression in keratin pearls only. __Conclusions__: This study validates the utility of CK13 IHC as a useful immunohistochemical marker in routine diagnostic practice to make distinction between non-neoplastic from dysplastic and neoplastic (malignant) oral lesions.

Keywords: Oral mucosa - dysplasia - carcinoma-in-situ - oral squamous cell carcinoma - cytokeratin 13

Introduction

Oral Squamous Cell Carcinoma (OSCC) is the leading cancer of the oral cavity. Both oral and pharyngeal cancers globally rank sixth among all the common cancers according to Warnakulasuriya (2009). In recent reports the burden of OSCC has been on the rise especially in South East Asia (Challacombe et al., 2011). Pakistan, Bangladesh, India, Nepal, and Sri Lanka have one of the highest incidences in the World. As per Karachi South Cancer Registry it is the 2nd most common cancer in both genders with a higher incidence in males according to Bhurgri (2005). By 2007 oral cancer surpassed lung cancer as the number 1 cancer in males of Karachi South (Bhurgri, 2007).

Worldwide the risk factors of OC include alcohol, tobacco, poor diet and high risk types of human papillomaviruses (Challacombe et al., 2011). However in sub-continent including Pakistan (Southern Pakistan) major risk factors are the alternate chewing habits i-e paan, gutka, naswar, areca nut, betel quid, paan masala etc. Smoking is also highly prevalent. This is also compounded by poor nutrition and oral hygiene (Bhurgri et al., 2003).

Clinically and microscopically precursor high risk lesions of OSCC are many such as leukoplakia, erythroplakia and oral submucousal fibrosis. Unfortunately most lack specific histological features unlike cervical precursor lesions in females (Wake, 1993; Warnakulasuriya et al., 2007). Biopsy and histologic examination of such lesions is essential to exclude precursor atypical lesions before transformation to invasive malignancy. A single ulcer, lump, red patches or white patches which persist for more than three weeks may be manifestation of malignancy or precursor high risk lesion and hence biopsy is required for histopathological assessment (Walsh et al., 2013).

Differential expression of various cytokeratins is extensively used in routine diagnostic pathology for precise categorization of epithelial malignancies (Moll et al., 1982; Moll, 1998). Cytokeratin 13 (CK13) is an intermediate filament protein which is immune-localized in the prickle cell layer and upper cell layers of oral mucosa. There is a loss of CK13 depending on the severity of dysplastic changes and transformation into malignancy. A total loss of CK13 in prickle cell layer of carcinoma in situ (CIS) can be considered as an important immunohistochemical marker for diagnosis of differentiated type of CIS which lack definite histological

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features of malignancy (Kobayashi et al., 2010).

Most OC in Pakistan are being diagnosed at advanced stages. Diagnosis at late stages results in high treatment costs, low affordability, poor treatment outcomes and increased mortality (Khandekar et al., 2006; David et al., 2008). Hence there is a need to diagnose and treat OC at early stages.

A significant number of oral lesion biopsies received, in our practice however show borderline (equivocal) features and on morphological grounds alone it is not possible to be sure if it is an atypical hyperplastic lesion or atypical neoplastic lesion. Such biopsies are commonly reported as 'Proliferative Squamous Lesions'.

Such non-committal reports are source of a common irritant for surgeons who are then stuck and cannot offer a definitive treatment plan to patients. Therefore, the objective of this study was to analyze the differential expression of CK13 on a cohort of oral mucosal biopsies including normal, hyperplastic (including atypical hyperplasia), dysplastic/CIS and invasive OSCC.

Materials and Methods

Sample Collection:

This cross sectional study was carried out on 93 consecutive oral biopsies (from cheek, tongue, floor of mouth & lip) collected during 2011-2014. These included nineteen (19) normal buccal mucosal, twenty one (21) hyperplastic oral mucosal, sixteen (16) severely dysplastic/CIS and thirty seven (37) invasive OSCC cases. The study was approved by the ethical review committee of the hospital. All biopsies were reviewed again by a senior pathologist with sub-specialty interest in oral cancers.

Immunostaining

Serial sections of 3-4μm were cut from the paraffin blocks. For immunohistochemistry, sections were mounted on glass slides coated with poly-L-lysine. The sections were deparaffinized with xylene and rehydrated in serial graded alcohol solutions and rinsed in deiodinized water. Target retrieval was performed with target retrieval solution in pre-heated water bath for 20 minutes at pH 9.0. Endogenous peroxidase activity was blocked by immersion in peroxidase solution for ten minutes and washed with Tris buffer saline with Tween 20. Sections were than treated with primary antibody against Monoclonal Mouse Anti-Human Cytokeratin 13, Clone DE-K13 (Dako Cytomation, Denmark) for 30 minutes at room temperature followed by rinsing with TSBT buffer. Treatment with labeled polymer (labeled polymer-HRP anti-mouse) was done and bound antibody was detected using Envision + system HRP (DAB) (Dako Cytomation, Denmark according to manufacturer's instructions). Counterstaining was done with hematoxylin and sections were mounted with cover slip for light microscopy.

Statistical analysis:

Statistical analysis was performed on SPSS version 20.0. For quantitative variables mean with standard deviation was calculated. For qualitative/categorical variables percentages and frequencies were calculated. Chi square was used to show association between Cytokeratin 13 and three categories. In all statistical analysis, p-value < 0.05 was considered to be significant. The statistical estimates are presented in the Table 1.

Results

Out of 93 biopsies, 70% were from males and 30% were from females. Their ages ranged from 20 years to 82 years with a mean of 48.2, 46.2% of the study subjects were less than 45 years of age while the age of 50.5% of participants was between 45 to 65 years and only 3.3% of participants were above 65 years of age.

All normal oral mucosal biopsies and unequivocal hyperplastic oral mucosal biopsies showed strong diffuse reactivity with CK13 immunohistochemical stain (Figure 1a&b, 2a&b). Biopsies diagnosed morphologically as severely dysplastic/CIS (differentiated type) also showed complete or near complete loss (Figure 3a & 3b). Only few biopsies out of 16 morphologically consistent with dysplasia/CIS showed focal weak reactivity. Likewise most cases of OSCC showed complete loss of CK13 expression (Figure 4a & 4b, 5a & 5b) except occasional focal reactivity in keratin pearls only. Based on these findings we found a strong statistical difference (p value=0.0001) of CK13 expression in normal and hyperplasia specimens as compared to dysplastic and OSCC specimens.
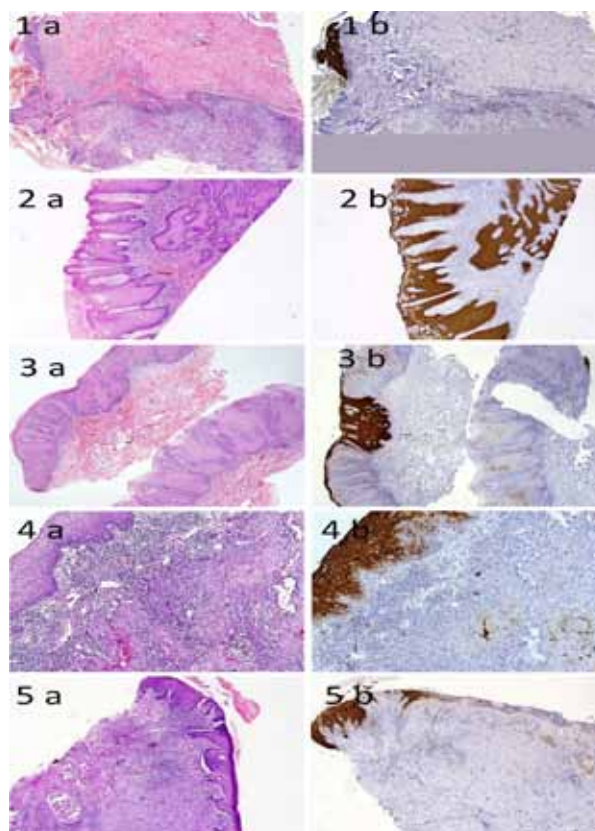

Figure 1. H&E Stain and CK13 Immunostaninig in 1. Normal, mucosal margin at upper left (1a,b); 2. Hyperplastic (2a,b); 3. Dysplastic/CIS (3a,b); 4. Subepithelial OSCC with overlying normal mucosa (4a, b); 5. Subepithelial OSCC with overlying normal & dysplastic mucosa (5a, b)

Table 1. : Immunoexpression of CK13 in Normal, Hyperplastic, Dysplastic/Ca in situ and Oral Squamous Cell Carcinoma Biopsies

		Strong diffuse positive	Extensive or near complete loss	Complete loss with focal reactivity in keratin pearls	Complete loss with focal strong reactivity in keratin pearls	Complete loss with focal weak reactivity in keratin pearls	Loss except in superficial granular layer	Complete loss	Total	p-value
Normal	N	19	0	0	0	0	0	0	19	0.0001
	%	100%	0%	0%	0%	0%	0%	0%	100%	
Hyperplasia	N	16	0	0	0	0	0	0	16	0.0001
	%	100%	0%	0%	0%	0%	0%	0%	100%	
Dysplasia / Ca in situ	N	0	4	4	0	0	1	7	16	0.0001
	%	0%	25%	25%	0%	0%	6.30%	43.80%	100%	
SCC	N	0	0	17	2	3	0	15	37	0.0001
	%	0%	0%	45.90%	5.40%	8.10%	0%	40.50%	100%	

SCC: Squamous cell carcinoma

In our study, few discrepancies between H&E diagnosis and CK13 immunohistochemistry were noticed. Five samples which were reported as atypical hyperplasia/ mild dysplasia on routine H&E staining exhibited loss of CK13. On review of H&E, they were relabeled as CIS (differentiated type).

Discussion

OC is the most common malignancy occurring in the head and neck region. The incidence of this cancer is rising in the younger age group. In our study about half of the patients were young (< 45 years). Cytokeratin 13 is an intermediate sized acidic type 1 protein and constitutes an important component of non- keratinized epithelia. It is a component of epithelia of oral mucosa, esophagus, anal canal, trachea, uterine cervix and urothelium (Moll et al., 1982; van et al., 1994; Itakura et al., 1996). In the present study CK13 was immunolocalized in the supra-basal layers of normal and hyperplastic oral epithelium but its expression gradually and finally completely disappeared as the tissue was transformed into severe dysplasia/ CIS and OSCC. Like this study some other studies also observed continuing loss of CK13 during the malignant transformation process (Mikami et al., 2011; Schaaij et al; 2010).These findings signify that there is a gradual IHC loss of CK13 during transformation of normal/ hyperplastic oral mucosal epithelium into dysplasia, CIS and invasive malignancy.

Some others have shown similar results with few exceptions. In a previous study it was found that there was atypical expression of CK13 in OSCC and oral epithelial dysplasia with concurrent up regulation of other keratins (Yanagava et al., 2007).

An important observation in our study was that some samples labeled as atypical hyperplasia /mild dysplasia on routine H&E staining showed loss of CK13 on immunohistochemistry so they were reviewed again and after expert opinion H&E initial diagnoses was changed. They were relabeled as CIS (Differentiated type).

Our study support that CK13 immunohistochemitry is very helpful in day to day evaluation of oral biopsies for precursor and neoplastic lesions. This may also prove valuable in evaluation of surgical margins. Loss of expression of cytokeratin 13 is usually also associated with increased expression of cytokeratin 17 (Noguchi et al., 2011). So a panel approach using CK13 and CK17 is even more useful in the precise diagnosis of Oral precursor and neoplastic lesions (Kitamura et al., 2012).

CK13 IHC loss in dysplastic and neoplastic lesions was also confirmed at genetic level. In some studies quantitative real-time PCR method was used to demonstrate considerable down- regulation of KRT13 gene in OSCC samples as compared with non-OSCC sample (Lallemant et al., 2009; Hiroko et al., 2012; Firstine et al., 2015).

In conclusion, Our study shows that CK13 IHC may be considered as a useful marker in the early diagnosis of dysplasia/CIS and OSCC as there is a gradual loss of Cytokeratin 13 expression in dysplastic and neoplastic oral mucosal epithelium. A total loss of CK13 in the prickle cell layer is an important immunohistochemical finding for diagnosis of precursor and malignant oral lesions.

Acknowledgements

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