J Oral Pathol Med (2006) 35: 25–32

www.blackwellmunksgaard.com/jopm

Cytokeratin expression in oral submucous fibrosis – an immunohistochemical study

K. Ranganathan[1], R. Kavitha[1], S. S. Sawant[2], M. M. Vaidya[2]

[1]*Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Uthandi, Chennai, India;* [2]*Department of Biochemistry and Cell Biology, Tata Memorial Center, Advanced Center for Treatment Research and Education in Cancer, Khargar, Mumbai, India*

BACKGROUND: Oral submucous fibrosis (OSF) is a pre-malignant condition caused by habitual use of areca nut, affecting the oro-pharynx and characterized by progressive fibrosis. Alteration of cytokeratin (CK) expression has been documented in leukoplakia and oral cancer (OC). However, very little is known of CK alterations in OSF. The present study was carried out to characterize the CK profile in OSF and ascertain if this could be used as a surrogate marker for malignant transformation.

METHODS: Paraffin-embedded tissues of OSF ($n = 50$), normal ($n = 10$) and OC ($n = 10$) were stained with pancytokeratin (PanCK), high molecular weight cytokeratin (HMWCK), CKs 18, 14, 8, 5, 4 and 1 by immunohistochemistry.

RESULTS: Significant difference in the CK staining pattern was seen between normal, OSF and cancer. Significant changes in OSF included increased intensity of staining for PanCK and HMWCK, aberrant expression of CK8 and decreased expression of CKs 5 and 14.

CONCLUSION: Cytokeratin profile of OSF was significantly different from normals for PanCK, HMWCK, CK8, 5 and 14 suggesting their potential to be used as surrogate markers of malignant transformation. Further studies will help in better defining the nature and clinical implications of these alterations.

J Oral Pathol Med (2006) **35**: 25–32

Keywords: areca nut; cancer; cytokeratin; immunohistochemistry; leukoplakia; oral; pre-cancer; submucous fibrosis

Introduction

Cytokeratin (CK) are epithelia specific intermediate filament proteins, that are broadly classified on the basis of their molecular weights and isoelectric points into two subfamilies: Type I: acidic with low molecular weight (CK9–23) and Type II: basic with high molecular weight (CK1–8) (1). They occur as heteropolymers that are expressed in specific pairs with one member from each subfamily (2). There are around 23 CK polypeptides expressed in human epithelia. Each type of epithelium expresses two to four specific pairs based on their differentiation status (3–5). As the CKs exhibit tissue specific expression they have been used as diagnostic markers in cancer and pre-cancer. The oral mucosa in humans express CK5 and CK14 on which other CK expression is superimposed depending on the keratinization pattern of epithelium (6). Buccal mucosa expresses CK4 and CK13 in addition to CK5 and CK14 while gingiva expresses CK1 and CK10 along with CK5 and CK14.

Oral submucous fibrosis (OSF) is a chronic, progressive, pre-cancerous condition of the oral mucosa, which is associated with areca nut chewing habit widely prevalent in India and South East Asia (7, 8). The condition is characterized by reduction of vasculature and submucosal fibrosis followed by epithelial atrophy. The altered epithelium has the potential for malignant conversion over a period of time. Oral pre-malignant lesions such as leukoplakia have been widely studied but the expression of CKs with respect to OSF has not been widely investigated. The present study, to our knowledge this is the first time that a wide array of CKs has been studied in OSF, was carried out to characterize the CK profile of OSF and compare it to those reported for pre-cancer (leukoplakia) and OC in the literature. This information would potentially be useful in identifying those OSF cases that have a higher risk of malignant conversion.

Materials and methods

Patients and tissues

Fifty cases of OSF, 10 each of normal and OC constituted the study material. The OSF cases for this study were collected from the patients attending the Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Chennai. OSF patients

Correspondence: Dr K. Ranganathan, Professor, Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, 2/102, East Coast Road, Uthandi, Chennai 600 119, India. Tel.: +91 44 244 91736. Fax: 91 44 244 93718. E-mail: ran2@vsnl.com
Accepted for publication August 8, 2005

were identified based on well-established clinical criteria (7, 9) and thorough history recorded in a pre-established format by trained dental surgeons. Patients with OSF were counseled with respect to the areca nut habit, and biopsies were taken after obtaining patients consent. Biopsies were taken from representative areas and the tissues were fixed in buffered formalin and embedded in paraffin. Hematoxylin and eosin staining was performed to confirm the clinical diagnosis. Fifty tissue blocks with sufficient tissue were taken for immunohistochemical staining.

Normal tissues were obtained from the buccal mucosa of 10 patients during the surgical removal of the third molar. Ten paraffin embedded blocks of OC of buccal mucosa (squamous cell carcinoma) were retrieved from the archives of the Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Chennai for this study. Immunohistochemical staining was carried out using commercially available monoclonal antibodies, listed in Table 1. Institutional Review Board and Ethical Committee approval was obtained prior to the start of the study. Informed consent was obtained from all patients.

Immunohistochemistry

Five micrometer sections of formalin fixed and paraffin embedded tissues were mounted on 3-aminopropyl tri ethoxysilane (APES) coated slides. Sections were dewaxed with xylene and rehydrated through a graded series of ethanol and finally in distilled water. Blocking was performed with 3% hydrogen peroxide in absolute methanol for 30 min to quench the endogenous peroxidase activity of the tissues followed by antigen retrieval with microwave treatment: microwave oven (Sharp R-4A52 BPL-Sanyo, India) at high for 3 min and at low for 5 min and finally allowing it to cool at room temperature for 20 min, in sodium citrate buffer (pH 6.0). The sections were then incubated with pre-immune horse serum for 30 min at room temperature to block the non-specific binding sites. The sections were then probed for the presence of different CKs using specific antibodies at appropriate dilutions (Table 1). The sections were stained by the modified labeled avidin-biotin technique with DAKO LSAB^{TM} kit (DAKO Corporation, Carpinteria, CA, USA) . The sections were stained with chromogen 3,3′ diaminobenzidine, counterstained with Harris hematoxylin and mounted. Tris-buffered saline of pH 7.0 was used throughout for washing and rinsing the slides. Positive and negative controls were included in all batches. To determine the specificity of the secondary antibody, the negative control was kept with tris-buffered saline instead of primary antibody. Normal epithelial tissues were used as positive controls.

Analysis of staining

The intensity of staining of the epithelium (basal and suprabasal) was assessed as: −, negative; +, mild; + +, moderate; + + +, intense. Two independent observers evaluated the slides (authors K.R. and R.K.). When discrepancy existed a third pathologist was asked to evaluate the slide to arrive at a consensus conclusion. The kappa statistics for the inter-observer variation was between 0.7–1. [PanCK: 0.93, high molecular weight CK (HMWCK): 0.9, CK1: 0. 88, CK4: 0.7, CK5: 0.9, CK8: 0.7, CK14: 0.94 and CK18: 0.96].

Statistical analysis

Data were entered and analyzed using SPSS 10.0.5 software. The chi-square test was used to analyze the differences between the intensity levels and percentage positivity in normal, OSF and OC for all CKs. Differences with a probability value of < 0.05 were considered statistically significant.

Results

Subjects

In the normal group there were six (60%) males and four (40%) females, in OC group there were five (50%) males and five (50%) females and in the OSF group there were 44 (88%) males and six (12%) females.

Staining results

Figure 1 shows representative photomicrographs of the staining results. Figure 1A1, A2 and A3 show hematoxylin and eosin stained sections of normal, OSF and OC, respectively.

Immunohistochemical staining

Pancytokeratin

Figure 1 shows representative photomicrographs of immunohistochemical staining for pancytokeratin

Table 1 Antibodies used in the immunohistochemical analysis for cytokeratins, with references for specificity

Antibody	*Manufacturer*	*Clone*	*Dilution*	*References (antibodies specificity)*
Pancytokeratin (1–8, 10, 13–16 and 19)	Dako	AE1/AE3	1:1*	Woodstock-Mitchell et al. (36), Tseng et al. (37)
High molecular weight cytokeratin (1, 5, 10 and 14)	Dako	34βE12	1:1*	Gown and Vogel (1982) (38), Gown and Vogel (1984) (39)
Cytokeratin 18	Sigma	CY90	1:800	Steimer et al. (40), Clark et al. (41), Muhlhauser et al. (42)
Cytokeratin 14	Sigma	CKB1	1:200	Kannan et al. (35)
Cytokeratin 8	Sigma	M20	1:200	Gown and Vogel (1982) (38), Gown and Vogel (1984) (39)
Cytokeratin 5	Novo Castra	XM26	1:100	Moll et al. (43)
Cytokeratin 4	Sigma	6B10	1:300	Van Muijen et al. (44), Leube et al. (45), Oosterwijk et al. (46)
Cytokeratin 1	Novo Castra	34βB4	1:20	Gown and Vogel (1982) (38)

*Ready to use.

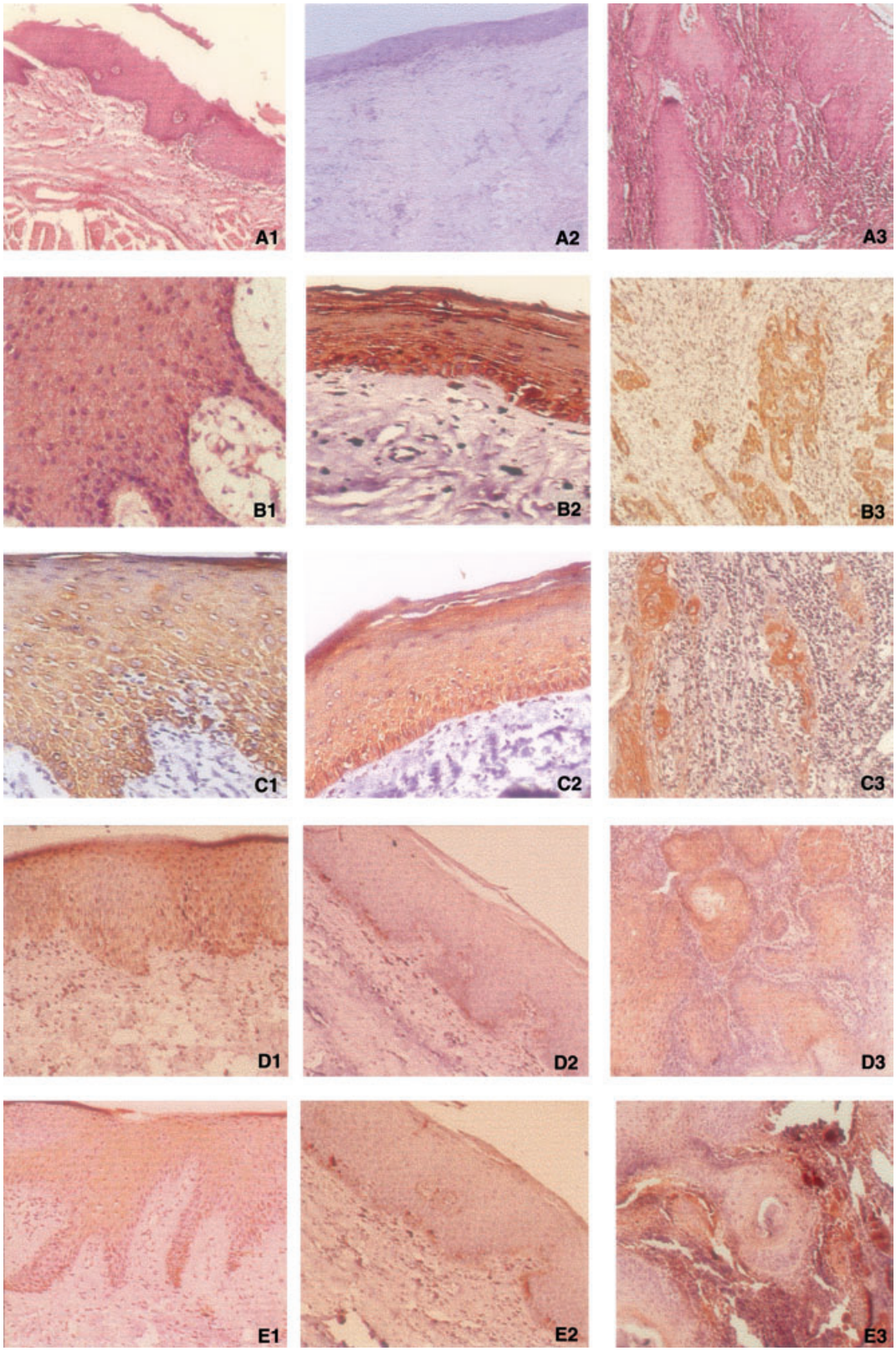

Figure 1 Representative photomicrographs of normal, OSF and OC. A: Hematoxylin and Eosin (H&E) stained sections; B, C, D, E, F, G, H, I: Immunohistochemical (IHC) staining for CKs. A1, A2, A3: H&E stained sections of normal, OSF and OC, respectively. B1, B2, B3: IHC for PanCK of normal, OSF and OC, respectively. C1, C2, C3: IHC for HMWCK of normal, OSF and OC, respectively. D1, D2, D3: IHC for CK18 of normal, OSF and OC, respectively. E1, E2, E3: IHC for CK14 of normal, OSF and OC, respectively. F1, F2, F3: IHC for CK8 of normal, OSF and OC, respectively. G1, G2, G3: IHC for CK5 of normal, OSF and OC, respectively. H1, H2, H3: IHC for CK4 of normal, OSF and OC, respectively. I1, I2, I3: IHC for CK1 of normal, OSF and OC, respectively.

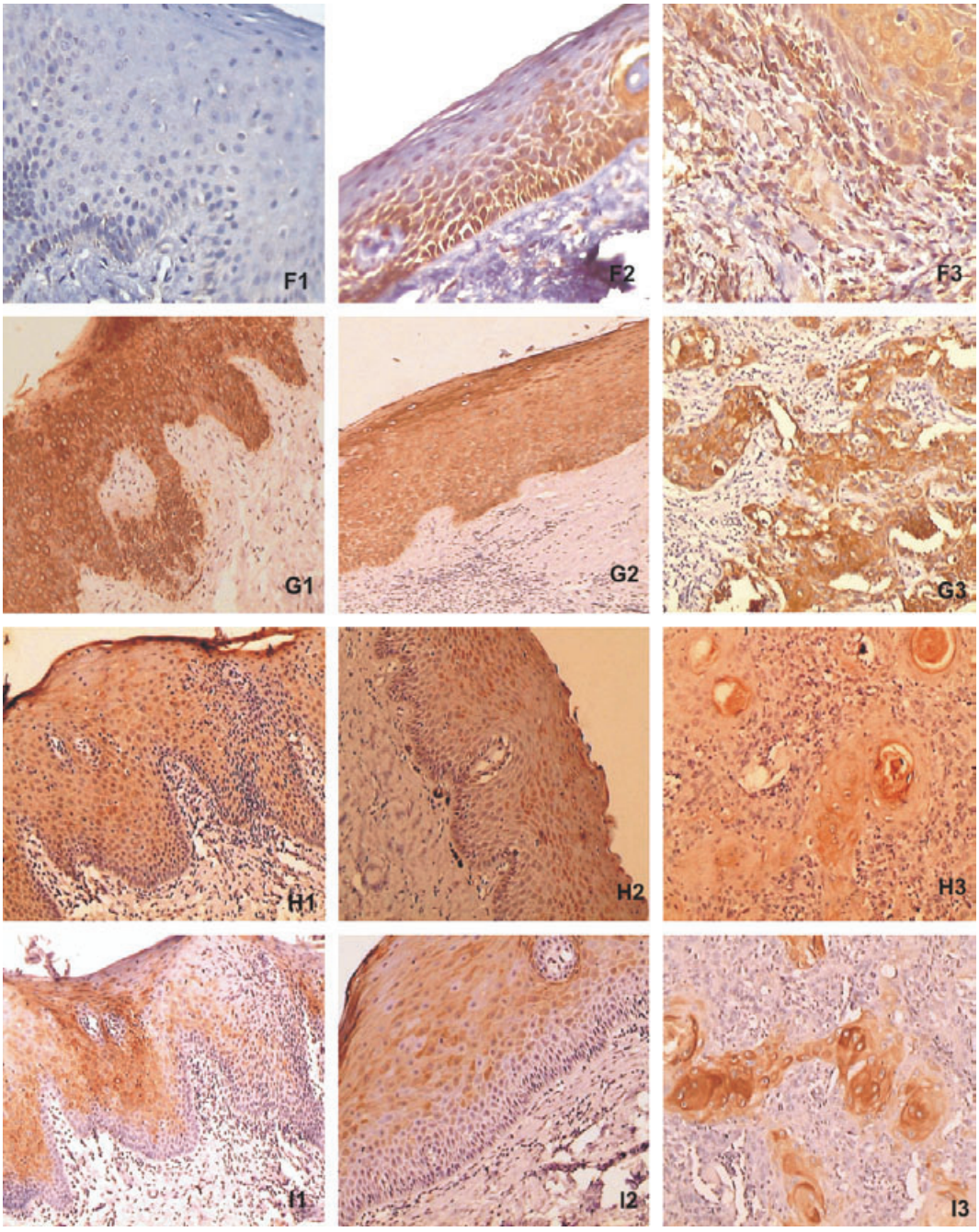

Figure 1 Continued.

(PanCK) in normal (B1), OSF (B2), and OC (B3). Staining is seen both basally and suprabasally in all the three groups.

All cases (100%) of normals, OSF and OC exhibited staining (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in normal seven (70%) and three (30%) showed mild and moderate intensity of staining, respectively, in OSF of 50 cases, 35 (70%) exhibited mild, 11 (22%) moderate and four (8%) intense staining and in OC the staining was moderate in six (60%) and intense in four (40%). The difference in the staining between the groups was statistically significant ($P = 0.00$).

High molecular weight cytokeratin

Figure 1 shows representative photomicrographs of immunohistochemical staining for HMWCK in normal (C1), OSF (C2), and OC (C3). Staining is seen both basally and suprabasally in all the three groups.

Staining was seen in all cases of OC (100%), nine cases (90%) of normal and 48 cases (96%) of OSF (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in normal seven (70%) cases exhibited mild while two (20%) exhibited moderate staining, in OSF 26 (52%) cases showed mild while 19 (38%) and three (6%) showed moderate and intense

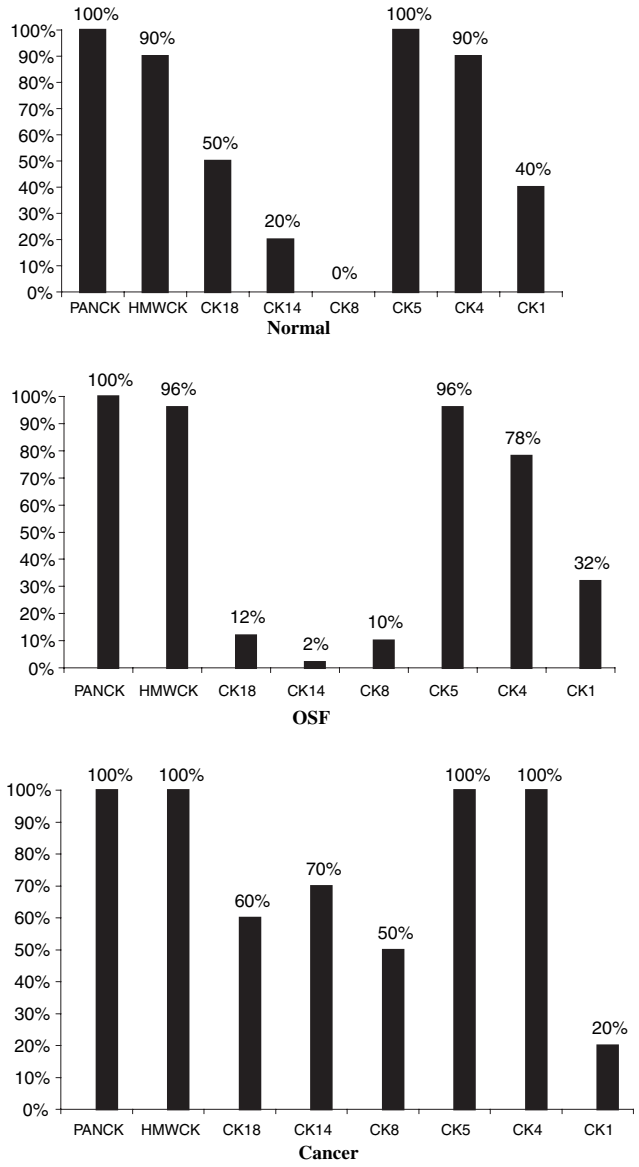

Figure 2 Comparison of percentage positivity of cytokeratins among the study groups.

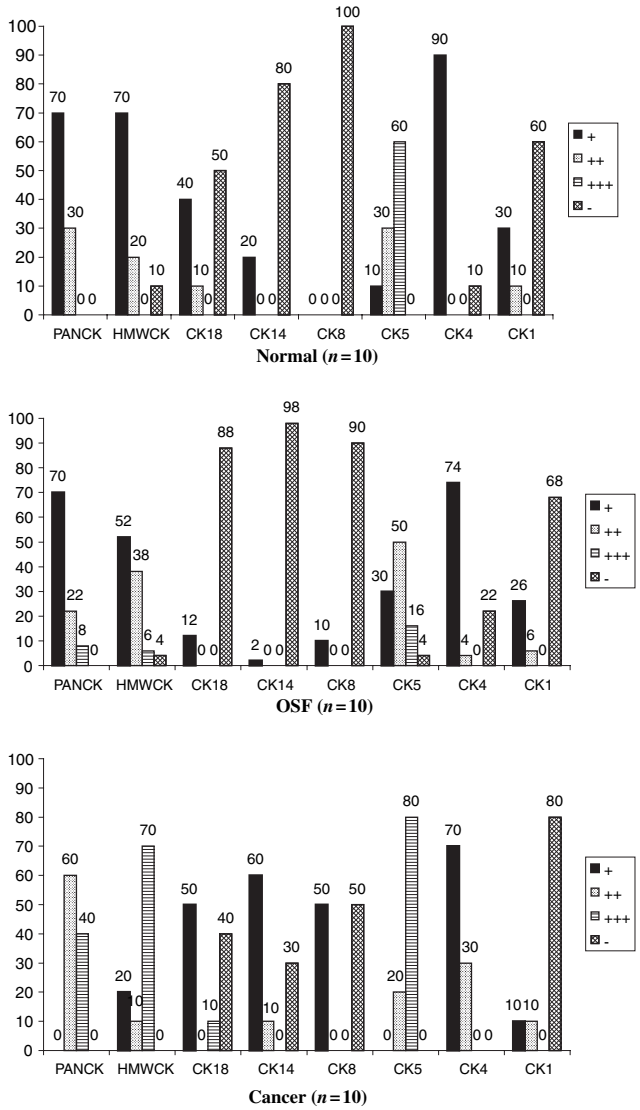

Figure 3 Comparison of intensity of staining of cytokeratins among the study groups.

staining, respectively and in OC seven (70%) of cases showed intense staining. The difference in the staining intensity between the three groups was statistically significant.

Cytokeratin 18

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK18 in normal (D1), OSF (D2), and OC (D3). The figure demonstrates basal staining in OSF and basal and suprabasal staining in normal and OC.

The CK18 expression was seen in five cases (50%) of normal, six cases (60%) of OC and six cases (12%) of OSF (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in normal four (40%) showed mild and one (10%) moderate intensity of staining, in OSF all the six (12%) cases that stained positive exhibited only mild staining and in OC five (50%) showed mild and one (10%) exhibited intense staining. The difference in expression was statistically significant between the three groups ($P = 0.00$).

Cytokeratin 14

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK14 in normal (E1), OSF (E2), and OC (E3). The figure demonstrates basal staining in OSF and basal and suprabasal staining in normal and OC.

The CK14 staining was seen in two cases (20%) of normals, one case (2%) of OSF and seven cases (70%) of oral cancers (OCs; Fig. 2). Histograms for the intensity of staining in Fig. 3 show that only mild staining was seen in normal and OSF, in OC six (60%) exhibited mild staining while one (10%) showed moderate staining. The expression in OSF was less than that in normals and OC ($P = 0.00$).

Cytokeratin 8

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK8 in normal (F1) OSF (F2), and OC (F3). No staining is seen in normal while basal and suprabasal staining is seen in OSF and OC.

The CK8 staining was not seen in any of the normal tissues studied while five (50%) of OC and five (10%) of OSF exhibited CK8 staining (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in OSF five (10%) showed mild staining while in OC all five (50%) showed mild staining. The difference between the three groups was statistically significant ($P = 0.00$).

Cytokeratin 5

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK5 in normal (G1), OSF (G2), and OC (G3). Staining is seen in both the basal and suprabasal layer in all three groups.

The CK5 staining was seen in all cases of normals and cancers and 48 (96%) of OSF (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in normal six cases (60%) showed intense staining as compared with eight cases (16%) of OSF and eight (80%) of OC, three (30%) of normal showed moderate staining compared with 25 (50%) of OSF and two (20%) OC and one (10%) and 15 (30%) exhibited mild staining in normal and OSF, respectively. In OC there were no cases showing mild staining. The difference in expression between the three groups was statistically significant ($P = 0.002$).

Cytokeratin 4

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK4 in normal (H1), OSF (H2), and OC (H3). Staining is seen in the basal and suprabasal layers in all the three groups.

The staining was positive in nine (90%) cases of normal, 39 (78%) of OSF and 10 (100%) cases of cancer (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that all the positive cases of the normals showed mild staining, in OSF 37 (74%) and two (4%) cases exhibited mild and moderate staining, respectively and in OC seven cases (70%) showed mild staining while three cases (30%) showed moderate staining intensity. There was a statistically significant difference ($P = 0.02$) between the three groups.

Cytokeratin 1

Figure 1 shows representative photomicrographs of immunohistochemical staining for CK1 in normal (I1), OSF (I2), and OC (I3), respectively. Staining is predominantly seen in suprabasal layer in normal and OSF and in basal and suprabasal layers in OC.

Four cases (40%) of normal, 16 cases (32%) of OSF and two cases (20%) of OC showed positive expression of CK1 (Fig. 2). Histograms for the intensity of staining in Fig. 3 show that in normal the three (30%) showed mild staining, in OSF 13 (26%) showed mild staining and in OC of the two (20%) showing staining there was one mild and one moderate staining.

Discussion

In India oral tumors constitute 30% of all malignancies and of these 90% are oral squamous cell carcinoma (10). Some of the OC arise from pre-existing pre-cancerous lesions such as OSF and leukoplakia. However, the malignant conversion rate of pre-cancerous lesion ranges from 8% to 10% (11). It is therefore important to identify markers that could help us to ascertain those lesions that have high potential for malignant conversion and treat them aggressively.

Alterations of CKs have been reported in OC and more recently in a few cases of OSF (12–14). It has been shown that changes in the underlying connective tissue are reflected in the adjacent epithelium and also result in alterations in CK expression (15). The expression of other CK pairs like 1 and 10 and CK4 and 13 is superimposed on this, depending on the differentiation and keratinization status of the tissue (6). CK expression has been shown to be altered under various pathological conditions like psoriasis, gingivitis, and hyperkeratosis (16–18). CK expression has also been shown to alter in oral pre-malignant conditions like leukoplakia and OSF and also in OC (6, 19–23). Two types of alterations have also been reported: (i) non-expression of certain basic CK and (ii) aberrant expression of some CK, which are not expressed by the normal tissue.

Non-expression of CK5 has been reported in some tumor derived cell lines. Sager (24) found down regulation of CK5 in breast cancer derived cell lines. Nan et al. (25) reported non-expression of CK5 in 12 cell lines derived from renal cell carcinomas, one of which was spontaneously transformed. Cell lines derived from normal tissues did express CK5. They concluded that this change seems to be associated with the process of carcinogenesis. Down regulation of 58.3 kDa keratins in severely dysplastic, 4NQO treated rat palatal mucosa has been shown by Nicholas et al. (26) Furthermore, they have shown loss of these two keratins – CK5 and 14 in SCC of rat palatal mucosa. Down regulation of basic CK pair of 5 and 14 has also been shown by Morgan et al. (6) in poorly differentiated SCC of oral mucosa.

Thr CK5 non-expression has been demonstrated in tobacco related oral carcinogenesis and in few cases of OSF (33.3%) (13, 27). Non-expression of CK5 mRNA has been demonstrated in oral leukoplakia, OSF and cancer of buccal mucosa (2). The CK pair 5 and 14 is normally expressed in all human oral epithelia. In our present study we show the absence of CK5 in four of 50 cases of OSF, while mild intensity of staining, suggestive of down regulation, of CK5 and 14 was seen in 30% and 2% of OSF respectively. Interestingly, the OCs in our study group did not show loss of CK5 staining; the probable reason for this could be the smaller sample size (12, 19). It would be relevant in future studies to ascertain if the cancers arising in OSF lesions retain the same alteration as seen in our present study. Consistent with the fact that CK5 and 14 are co-expressed together, there was decreased expression of CK14 in OSF group compared with normal and OC.

The CKs 8 and 18 are co-expressed predominantly in simple epithelium (1). Aberrant expression of CK8 has also been shown in leukoplakia (13). In the present study CK8 expression was absent in normal samples and showed increased expression in OSF and OCs. CK18 expression was seen in OSF samples as well as in OC tissues. Some of the normal tissues also showed CK18 expression. As these samples were collected from patients who had come for tooth extraction and did not have good oral hygiene, it is possible that these tissues were not fully normal. Thus aberrant expression of CK18 in these tissues could be indicative of initiation of abnormal cell differentiation. Although CK18 was detected six OSF samples, its partner CK8 was not detected in one of them. This probably could be the result of deregulation of paired expression in human oral pre-cancer (13, 27, 28). Further studies on the mechanism of deregulation of paired expression should give us more information with respect to this anomalous expression.

Various groups have shown that some simple epithelial CK like CK8 and 18 are expressed in cancer of oral mucosa (29–31). More recently it has been shown that the transfection of CK8 gene can alter the phenotypic characteristic of fetal buccal cell lines leading to malignant transformation (32). Transgenic mice expressing human CK8 in the epidermis has been shown to exhibit severe epidermal and hair follicle dysplasia with concomitant alteration in epidermal differentiation markers (33). CKs are also involved in cell signaling response to stress and apoptosis (34). These findings suggest that not only is CK expression altered in oral carcinogenesis, but they may also play a contributory role in oral carcinogenesis (2, 32).

Studies have shown anomalous expression of CK1 and /or CK10 in well-differentiated carcinoma of buccal mucosa and down regulation of CK1 and 10 in the suprabasal layers of both pre-cancerous lesions and cancer (28, 33, 35). There was a decrease in CK1 positivity from normal to OSF to cancer. Also, HMWCK which is a cocktail of CK1,10; and 5,14 did show statistically significant increase in the intensity of staining in OSF and OC compared with normals, this could represent either alteration in CKs 5 and 14 or 1 and 10.

Pancytokeratin (CK1,10; 4,13; 5,14; 6,16; 7,19; 2,3; and 8,15) also showed a statistically significant increased intensity of staining from normal to OSF to cancer, although the percentage positivity was the same.

Our results suggest that there are gross alterations accruing in CK expression in OSF. In case of tobacco related cancers the oral cavity is continually exposed to various traumas due to the effect of thermal, mechanical and chemical stimuli, which when accompanied by inflammatory states may promote the growth of neoplastic changes. This probably results in changes in both oral mucosa and the underlying connective tissue, (15) which is reflected in alterations in CK expression pattern.

In conclusion OSF in the present study does show a statistically significant decreased expression of CK5 and 14, aberrant expression of CK8. Both PanCK and HMWCK also exhibited an increased intensity of staining from normal to OSF to OC. These findings suggest that in the epithelium in OSF alterations of CKs occur, similar to that seen in pre-cancerous lesions and OC. These alterations may have a role in carcinogenesis and have the potential to be used as surrogate markers of malignant transformation.

References

1. Moll R, Franke WW, Schillen D, Gelger B, Kepler H. The catalog of human cytokeratin: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982; **31**: 11–24.
2. Eichner R, Sun TT, Achi U. The role of keratin subfamilies and keratin pairs in the formation of human epidermal intermediate filaments. *J Cell Biol* 1986; **102**: 1767–77.
3. Franke WW, Schmid E, Schiller Dl, et al. Differentiation related patterns of expression of proteins of intermediate filaments in tissues and cultured cells. *Cold Spring Harbor Symp Quant Biol* 1982; **46**: 431–53.
4. Clausen H, Vedtofie P, Moe BD, Dabelsteen B, Sun TT, Dale B. Differentiation dependent expression of keratins in human oral epithelia. *J Invest Dermatol* 1986; **86**: 249–54.
5. Van Muijen GNP, Warner SD, Ponec M. Differentiation related changes of cytokeratin expression in cultured keratinocytes and in fetal newborn and adult epidermis. *Exp Cell Res* 1987; **171**: 331–46.
6. Morgan PR, Shirlow PJ, Johnson NW, Leigh IM, Lane EB. Potential applications of anti-keratin antibodies in oral diagnosis. *J Oral Pathol* 1987; **16**: 212–22.
7. Neville BW, Damm DD, Allen CM, et al. (eds). *Oral and Maxillofacial Pathology*, 2nd edn. USA: W.B. Saunders Publication, 2002; 349–50.
8. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special reference to the role of areca nut-chewing. *J Oral Pathol Med* 1995; **24**: 145–52.
9. Ranganathan K, Umadevi M, Joshua E, Bharadwaj A, Rooban T, Viswanathan R. Mouth opening, cheek flexibility and tongue protrusion parameters of 800 normal patients in Chennai, South India – A baseline study to enable assessment of alterations in oral submucous fibrosis. *J Indian Dent Assoc* 2001; **72**: 78–80.
10. Jussawalla DJ, Yeole BB, Natekar MV. *Cancer incidence in greater Bombay 1973–78,* "an epidemiological study". Bombay: Indian Cancer Society, 1985.
11. Daftary DK, Murti PR, Bhonsle RR, Gupta PC, Mehta FS, Pindborg JJ. Risk factors and markers for OC in high-risk areas of the world. In: Johnson NW, ed. *OC: The detection of patients and lesions at risk*. Cambridge (UK): Cambridge University Press, 1991; 29–63.
12. Sawant SS, Naresh KN, D'Cruz A, Ogale SB, Vaidya MM. Demonstration of cytokeratin-5 expression in tobacco related oral carcinogenesis-use of reverse transcriptase polymerase chain reaction as a sensitive assay. *Oral Oncol* 2003; **39**: 789–95.
13. Vaidya MM, Sawant SS, Borges AM, Ogale SB, Bhisey AN. Cytokeratin expression in precancerous lesions of the human oral cavity. *Eur J Cancer B Oral Oncol* 1998; **34**: 261–4.
14. Ojaswani S, Upasani OS, Milind M. Vaidya, Bhisey AN Database on monoclonal antibodies to cytokeratins. *Oral Oncol* 2004; **40**: 236–56.

15. Mackenzie IC, Hill MW. Maintenance of regionally specific patterns of cell proliferation and differentiation in transplanted skin and oral mucosa. *Cell Tissue Res* 1981; **219**: 597–607.
16. Skerrow D, Hunter I. Protein modifications during the keratinisation of normal and psoriatic human epidermis. *Biochem Biophys Acta* 1978; **537**: 474–84.
17. Bosch FX, Ouhayoun JP, Bader BL, et al. Extensive changes in cytokeratin expression patterns in pathologically affected human gingiva. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1989; **58**: 59–77.
18. Ogden GR, Chisholm DM, Leigh IM, Lane EB. Cytokeratin profiles in dyskeratosis congenita: an immunocytochemical investigation of lingual hyperkeratosis. *J Oral Pathol Med* 1992; **21**: 353–7.
19. Lindberg K, Rheinwald JG. Suprabasal 40 kD keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. *Am J Pathol* 1989; **134**: 89–98.
20. Ermich T, Schulz J, Raabe G, Schmann D. Pattern of oral cytokeratins. III. SDS- electrophoretic analysis and immunoblotting of cytokeratins in leukoplakias and squamous cell carcinomas of the oral mucosa. *Biomed Biochim Acta* 1989; **48**: 393–401.
21. Vigneswaran N, Peters KP, Hornstein OP, Haneke E. Comparison of cytokeratin, filaggrin and involucrin profiles in oral leukoplakias and squamous carcinomas. *J Oral Pathol Med* 1989; **18**: 377–90.
22. Heyden A, Huitfeldt HS, Koppang HS, Thrane PS, Bryne M, Brandtzaeg P. Cytokeratins as epithelial differentiation markers in premalignant and malignant oral lesions. *J Oral Pathol Med* 1992; **21**: 7–11.
23. Schulz J, Ermich T, Kasper M, Raabe G, Schumann D. Cytokeratin pattern of clinically intact and pathologically changed oral mucosa. *Int J Oral Maxillofac Surg* 1992; **21**: 35–39.
24. Sager R. Tumor suppressor genes: the puzzle and the promise. *Science* 1989; **246**: 1406–12.
25. Nan L, Kawamata H, Tan X, Kameyama S, Oyasu R. Differential expression of keratin 5 gene in non-tumorigenic and tumorigenic rat bladder cell lines. *Cancer Lett* 1993; **75**: 87–93.
26. Boyd NM, Reade PC. Temporal alterations in cytokeratin expression during experimental oral mucosal carcinogenesis. *Carcinogenesis* 1991; **12**: 1767–71.
27. Vaidya MM, Borges AM, Pradhan SA, Bhisey AN. Cytokeratin expression in squamous cell carcinomas of the tongue and alveolar mucosa. *Eur J Cancer B Oral Oncol* 1996; **32B**: 333–6.
28. Vaidhya MM, Borges AM, Pradhan SA, Rajpal RM, Bhisey AN. Altered keratin expression in buccal mucosal squamous cell carcinoma. *J Oral Pathol Med* 1989; **18**: 282–6.
29. Ogden GR, Chisholm DM, Adi M, Lane EB. Cytokeratin expression in oral cancer and its relationship to tumor differentiation. *J Oral Pathol Med* 1993; **22**: 82–86.
30. Kannan S, Balaram P, Chandran GJ, et al. Differential expression of cytokeratin proteins during tumor progression in oral mucosa. *Epithelial Cell Biol* 1994; **3**: 61–69.
31. Morgan PR, Su L. Intermediate filaments in oral neoplasia. I. Oral cancer and epithelial dysplasia. *Eur J Cancer B Oral Oncol* 1994; **30**: 160–6.
32. Raul U, Sawant S, Dange P, Kalraiya R, Ingle A, Vaidya M. Implications of cytokeratin 8/18 filament formation in stratified epithelial cells: Induction of transformed phenotype. *Int J Cancer* 2004; **111**: 662–8.
33. Casanova ML, Bravo A, Martinez-Palacio J, et al. Epidermal abnormalities and increased malignancy of skin tumors in human epidermal keratin 8-expressing transgenic mice. *FASEB J* 2004; **18**: 1556–8.
34. Coulombe PA, Omary MB. "Hard'' and "Soft'' principles defining the structure, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* 2002; **14**: 110–22.
35. Kannan S, Balaram P, Chandran GJ, et al. Alterations in expression of terminal differentiation markers of keratinocytes during oral carcinogenesis. *Pathobiology* 1994; **62**: 127–33.
36. Woodstock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. *J Cell Biol* 1982; **95**: 580.
37. Tseng SCG, Jarvinen MJ, Nelson WG, Huang JW, Woodcock-Mitchell J, Sun TT. Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. *Cell* 1982; **30**: 361.
38. Gown AM, Vogel AM. Monoclonal antibodies to intermediate filament proteins of human cells: unique and cross-reacting antibodies. *J Cell Biol* 1982; **95**: 414.
39. Gown AM, Vogel AM. Monoclonal antibodies to human intermediate filament proteins II. Distribution of filaments in normal human tissues. *Am J Pathol* 1984; **114**: 309.
40. Stiemer B, Graf B, Neudeck H, Hildebrandt R, Hopp H, Weitzel HK. Antibodies to cytokeratins bind to epitopes in human uterine smooth muscle cells in normal and pathological pregnancies. *Histopathology* 1995; **27**: 407–14.
41. Clark MA, Wilson C, Sama A, Wilson JA, Hirst BH. Differential cytokeratin and glycoconjugate expression by the surface and crypt epithelia of human palatine tonsils. *Histochem Cell Biol* 2000; **114**: 311–21.
42. Muhlhauser J, Crescimanno C, Kasper M, Zaccheo D, Castellucci M. Differentiation of human trophoblast populations involves alterations in cytokeratin patterns. *J Histochem Cytochem* 1995; **43**: 579–89.
43. Moll R, Dhouailly D, Sun TT. Expression of keratin 5 as a distinctive feature of epithelial and biphasic mesotheliomas: an immunohistochemical study using monoclonal antibody AE14. *Virchows Archiv B Cell Pathol* 1989; **58**: 129–45.
44. Van Muijen GN, Ruiter DJ, Franke WW, et al. Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. *Exp Cell Res* 1986; **162**: 97–113.
45. Leube RE, Bader BL, Bosch FX, Zimbelman R, Achtstaetter T, Franke WW. Molecular characterization and expression of the stratification related cytokeratins 4 and 15. *J Cell Biol* 1988; **106**: 1249–61.
46. Oosterwijk E, Van Muijen GN, Oosterwijk-Wakka JC, Warnaar SO. Expression of intermediate- sized filaments in developing and adult human kidney and in renal cell carcinoma. *J Histochem Cytochem* 1990; **38**: 385–92.

Acknowledgments

This study was supported by Department of Biotechnology, Ministry of Science and technology, Government of India, Sanction number BT/PR/1592/MED/09/249/99.

We acknowledge Dr R.Viswanathan, Dr T.R. Saraswathi, Mrs R. Hemalatha, Dr S. Ramachandran, Dr R. Gunaseelan, Ragas Dental College and Hospital and Dr Zingde, ACTREC for their advise and assistance during this study.