Original Article

The importance of ctokeratins in the early detection of oral squamous cell carcinoma

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Abstract

Background: Oral cancer is usually diagnosed at advanced stages. The pattern of keratin expression in normal epithelia and the change in their expression in premalignant lesions and carcinomas have suggested the possibilities of improving diagnosis. The aim of this study is to determine the use of acidic cytokeratins (CKs) as biomarkers of histopathological progression in oral carcinogenesis.

Materials and Methods: A total of 50 paraffin blocks of histological specimens diagnosed as hyperplastic epithelium, dysplastic epithelium, well-differentiated squamous cell carcinoma (SCC) and poorly-differentiated SCC (10 specimens each) were included in this study, in addition to 10 normal oral mucosal samples. All samples were stained immunohistochemically with CKs (10-ab1, 14, 16-ab1, 18-dc10 and 19-abs10) using Ventana Medical Systems (Arizona-USA). The expression of CKs antigen was evaluated as absent, mild, moderate and severe.

Results: CK10-ab1 was found to be positive in the suprabasal layers of all specimens in normal and hyperplastic epithelium, while it was moderate in dysplastic epithelium and mild in well-differentiated SCC. CK10-ab1 was negative in all samples with poorly-differentiated SCC ($P < 0.005$). CK14 was positive in all specimens of all groups whereas CK16-ab1 was negative in all specimens of all groups. The stain of CKs 18-dc10 and 19-abs10 was restricted to the basal cells only in normal, hyperplastic and dysplastic epithelium, while it was mild in well-differentiated and poorly-differentiated SCC ($P < 0.01$).

Conclusion: CK10-ab1 disappeared gradually with the progression of malignant changes of squamous cells whereas CKs 18-dc10 and 19-abs10 increased gradually at the same time. Such changes in the protein mapping of squamous cells need more investigation for a better understanding of oral SCC.

Keywords: Cytokeratins, epithelial dysplasia, malignant transformation, oral squamous cell carcinoma

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INTRODUCTION

Around 40% of all head-and-neck malignancies[1] and more than 90% of all oral malignancies are recognized to be squamous cell carcinomas (SCCs). Oral cancer is a very significant contributor to the overall international cancer rate especially in the developing countries.[1] It has been reported that oral cancer is ranked as 8th and 16th most commonly occurring cancer in developing and developed countries, respectively,[2] being diagnosed usually at the advanced stages (III or IV), thus making it a global health problem.

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In the literature, several studies have attempted to investigate the factors behind the diagnostic delay of oral SCC (OSCC) and have also tried to explore reasons behind inconsistency in the figures about prevention and early detection of oral cancer over recent decades. Lack of awareness of the general population regarding the signs, symptoms and risk factors of oral cancer, as well as deficiency of prevention and early detection by healthcare professionals, is both believed to be accountable for the delay of its diagnosis.[3,4]

It is a well-recognized fact that OSSCs are headed by evident changes in the oral mucosa, usually in the form of appearance of white patches (leukoplakia) and red patches (erythroplakia).[1] The early diagnosis and treatment of these early intraepithelial oral lesions are highly substantial as it leads to high survival rates of OSSC patients.[5]

It has been emphasized by the International Agency for Research on Cancer and the World Health Organization that the effective planning for cancer control and screening strategies can lead to a reduction in one-third of the predicted 15 million cancer cases in future and also another third can be effectively managed.[6] Development of education programs and screening measures for early diagnosis of oral cancer can provide an opportunity to improve the prognosis of oral cancer patients in developing countries.[7]

Immune histochemical examination using specific markers such as cytokeratins (CKs) has been suggested for detection of oral cancer. CKs are intermediate protein filaments forming the skeleton of the epithelial cell cytoplasm. It exhibits the distinct pattern of expression in specific epithelial tissues.[8] In stratified epithelium, differentiating epithelial cells produce a pattern of diverse keratins as they migrate toward the outer layers.[9,10]

Keratins have a number of specific benefits as marker proteins. They are numerous, highly stable and extremely antigenic. Antibodies to groups of keratins are, therefore, extensively used as cellular markers of several epithelial and their corresponding neoplasms.[11]

CK10 is believed to be a particular marker of terminal differentiation of the keratinocytes.[12] It was immunohistochemically witnessed only in superficial layers of epithelia.[13] Expression of CKs 10, 13 and 14 was reported to have changed in some oral autoimmune lesions.[14] Keratin 10 was also found to be immunopositive in oral keratotic dysplasia.[15] CK2 has been reported to be an essential and adequate binding partner of CK10 at distinct body sites of mouse. Loss of CK2 expression causes aberrant aggregation of CK10, hyperkeratosis and inflammation.[16]

Differences in expression of CK19 can be utilized as a diagnostic tool in differentiating between odontogenic keratocysts and dentigerous cysts,[17] while the expression pattern of CK14 may provide an insight into the histogenesis of adenomatoid odontogenic tumor.[18] On the other hand, the upregulations of CK19 propose its possible use in fine-needle aspiration biopsy based on the preoperative diagnostics of cystic thyroid lesions.[19] Another study showed that CK profile could be used to guide the detection of cellular types and differential diagnosis of salivary gland tumors.[20]

Regarding CK expression in carcinomatous tissues, most carcinoma tissues exhibited de novo expression of five CKs (CKs 7, 8, 18, 10 and 17) and CK19 was detected both in basal and suprabasal positions. Certain CKs (CKs 10 and 19) proved to be significantly correlated to tumor size.[21]

da Silveira *et al*.[22] linked the expression of CK10 with disease outcome (death/remission), presence of metastases, clinical staging and histological grade. Positivity was found inversely connected with the incidence of metastases in cervical lymph nodes in their study. They concluded that biologically, the occurrence of this protein might impair modifications in the epithelial differentiation program of neoplastic cells, preventing these cells from attaining the capacity to separate from the primary tumor, thus initiating metastases.

CK19 might be associated with the clinical progression and differentiation of OSCC, and CK20 could be related to metastases of neck lymph nodes in OSCC. Due to the significant upregulation and the strong overexpression, CK17 could be the most appropriate marker for diagnosis of OSCC in the CK-family.[23] Recently, Frohwitter *et al*.[24] studied the expression of low-molecular-weight and high-molecular-weight CKs and correlated it with the pathogenetic pathways in oral SCCs.

The pattern of keratin expression in normal epithelia and the change in their expression in premalignant lesions and carcinomas have suggested the possibilities of improving diagnosis.[8,25] This stimulated the research to study the expression changes of selected acidic CKs' members in normal oral mucosa, hyperplastic, dysplastic and malignant squamous stratified epithelium (SSE).

MATERIALS AND METHODS

A total of 50 paraffin blocks of histological specimens diagnosed as hyperplastic epithelium, dysplastic epithelium, well-differentiated SCC and poorly-differentiated SCC (10 specimens each) were collected from the university referral hospital in the Eastern Province (KSA), in addition to 10 normal oral mucosal samples taken from the patients who come to the dental clinics in the College of Dentistry, Dammam University for surgical extractions after signing an informed consent. To unify samples and to minimize bias, all samples included in this study were taken (or originated) from keratinized SSE.

Basic histological preparation

Paraffin blocks were collected from all the groups. Five micron sections were prepared from each block. Thereafter, the sections were deparaffinized and processed for routine Hematoxylin and Eosin staining to confirm the diagnosis.

Immunohistochemical analysis

Five micron thick sections were deparaffinized and immersed in methanol with 3% hydrogen peroxidase for 5 min to eliminate endogenous peroxidase activity. For antigen retrieval, sections used for CKs antigen immunostaining were first autoclaved at 121°C in citrate buffer (pH 6.0) for 10 min. Ventana Medical Systems (Arizona-USA) were used for staining with corresponding buffer ethylenediaminetetraacetic acid 8.0 from the same company. Samples were then be incubated for 32 min. Finally, the sections were briefly counterstained with hematoxylin and prepared for evaluation. The following CKs were used with each specimen CKs (10-ab1, 14, 16-ab1, 18-dc10 and 19-abs10).

Microscopic evaluation

Light microscope was used for microscopic evaluation. Brown staining was regarded as being CK positive. The number of stained and nonstained cells was recorded separately and coded as:

1. Absent (−): 0% of squamous cells stained
2. Mild (+): 1%–25% of squamous cells stained
3. Moderate (++): 26%–50% of squamous cells stained
4. Severe (+++): More than 50% of squamous cells stained.

To determine the percentage of positively stained cells, each specimen was evaluated by counting all cells in fields of full-thickness epithelium under magnification of (×20) until at least 500 cells had been counted. With regard to well- and poorly-differentiated SCCs, three different diagnostic fields were chosen to count the cells.

Ethical approval

Research was approved by the Ethical Committee of the University of Dammam with approval number (201135).

Statistical analysis

Data analysis was performed using SPSS Version 20 (IBM Corporation, Chicago, USA). Diagnostic results were categorized on the basis of proportions of positive cells out of total 500 cells. Thus, the results were presented in terms of frequency and percentages. Chi-square test was applied to compare the proportion of similar and variant diagnostic results based on the markers between control and confirmed positive groups. Using the actual number of positive cells for each group following different diagnostic markers, receiver-operating curve (ROC) was established to find the diagnostic yield of markers used in this study. Area under the ROC = 0.70 was considered acceptable diagnostic field for a marker. $P \leq 0.05$ was considered statistically significant.

RESULTS

Among the 50 cases studied, 24 (48%) were female and 26 (52%) were male. The mean age detected was 55.2 years. All specimens of group control (normal oral epithelia) showed severe expression (+++) of CK10-ab1 (100%) in the suprabasal layers. Similarly, all specimens taken from lesions with hyperplastic epithelia showed severe expression (+++) of CK10-ab1 (100%) of squamous cells. With regard to dysplastic epithelium, 4 (40%) had severe expression (+++) of CK10-ab1, 4 (40%) had moderate expression (++) and 2 (20%) of them had mild expression (+). Of the 10 well-differentiated SCCs, expression of CK10-ab1 appeared in scattered cells only (mild stain 14% of counted cells). However, the expression of CK10-ab1 was negative (−) in all poorly-differentiated SCCs. Statistical differences were found to be significant ($P < 0.05$) [Table 1]. Results of this study showed gradual disappearance of CK10-ab1 with the progression of SCC [Figures 1 and 2].

CK14 was found to be positive (+++) in all squamous cells of all cases (100%), while CK16-ab1 was found to be negative (−) in all layers of all specimens with no statistical differences among different groups for both markers. CK8-dc10 was found to be positive in the basal layers only with the negative stain of squamous cells 0% (−) in all specimens in normal, hyperplastic and dysplastic epithelium. Scattered squamous cells (7%) and (17.2%) were positive (+) stained in well- and poorly-differentiated SCC,

Table 1: Expression of different markers in control group compared with all other study groups

Group	Control (*n*=10)				Hyperplastic (*n*=10)				*P*
	+++	++	+	–	+++	++	+	–	
CK 10ab1	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0.999
CK 14	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0.999
CK 16ab1	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 18dc10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 19abs10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
Group	**Control (*n*=10)**				**Dysplastic (*n*=10)**				***P***
	+++	++	+	–	+++	++	+	–	
CK 10ab1	10 (100)	0 (0)	0 (0)	0 (0)	4 (40)	4 (40)	2 (20)	0 (0)	0.014
CK 14	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0.999
CK 16ab1	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 18dc10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 19abs10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
Group	**Control (*n*=10)**				**Well-differentiated SCC (*n*=10)**				***P***
	+++	++	+	–	+++	++	+	–	
CK 10ab1	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0.001
CK 14	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0.999
CK 16ab1	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 18dc10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0.001
CK 19abs10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0.001
Group	**Control (*n*=10)**				**Poorly-differentiated SCC (*n*=10)**				***P***
	+++	++	+	–	+++	++	+	–	
CK 10ab1	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (100)	0.001
CK 14	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0.999
CK 16ab1	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0.999
CK 18dc10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	2 (20)	8 (80)	0 (0)	0.001
CK 19abs10	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	2 (20)	8 (80)	0 (0)	0.001

Statistical differences and *P* value are present in the last column. Absent (-): 0% of squamous cells stained, Mild (+): 1%-25% of squamous cells stained, Moderate (++): 26%-50% of squamous cells stained, Severe (+++): >50% of squamous cells stained. SCC: Squamous cell carcinoma

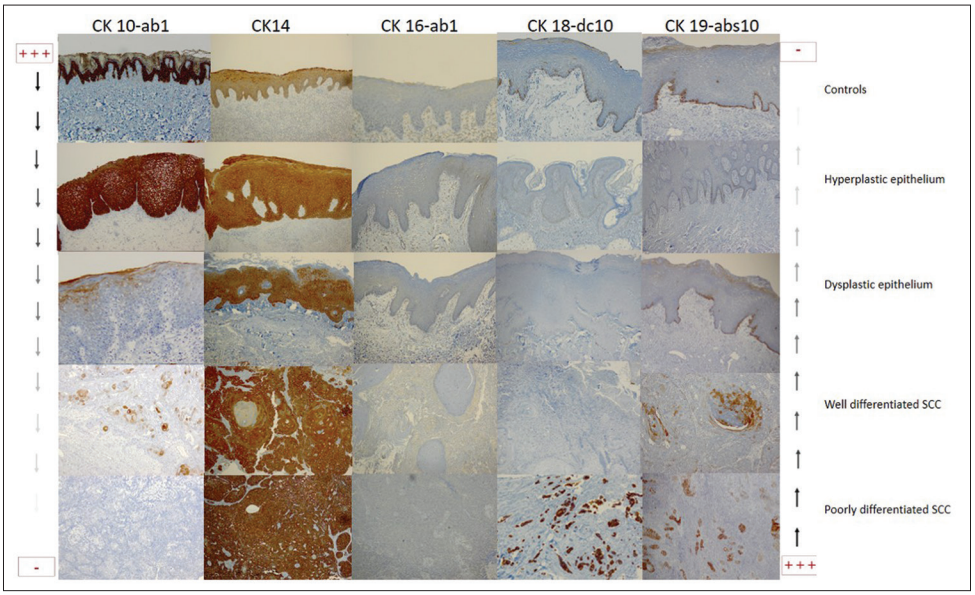

Figure 1: Panoramic microscopic photograph showing the expression of different cytokeratins among normal squamous stratified epithelium, hyperplastic squamous stratified epithelium, dysplastic squamous stratified epithelium and malignant squamous stratified epithelium

respectively. Similarly, CK19-abs10 was found to be positive in the basal layers in all specimens of normal, hyperplastic and dysplastic epithelium. However, scattered squamous cells (9.8%) and (17.8%) were positive (+) stained in well- and poorly-differentiated SCC, respectively [Figure 1].

DISCUSSION

Early detection of OSCC has a great impact on improving long-term patient's outcome. Different methods are used, one of which is oral visual screening is an effective method

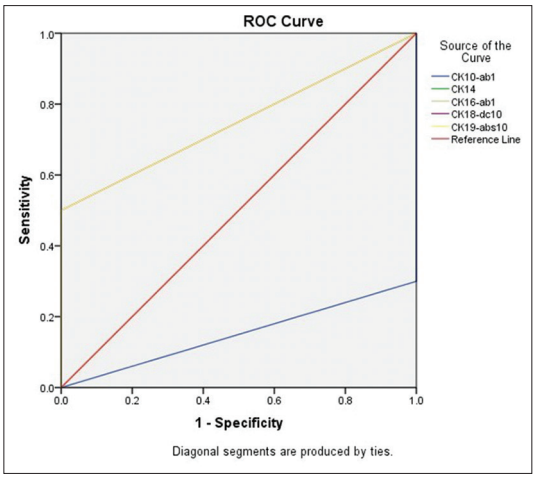

Figure 2: Diagnostic yield of markers was found acceptable on the basis of area under the curve (0.750)

in reducing mortality in high-risk individuals.[26] Moreover, immune-histochemical examination using specific cell markers, such as CKs, is another approach used for detection of oral cancer. The aberrant expression of CKs as a family was suggested as a prognostic indicator.[25]

The aim of this study is to correlate early malignant changes in the squamous cells with the expression of different acidic CKs.

Heyden *et al*.[27] reported that keratin pair 1/10 represents differentiating squamous cells; these results are in agreement with this study where CK10 was expressed strongly in 100% of squamous cells. In addition, the results of this study showed that the expression of CK10 disappears gradually along with the dysplastic changes of squamous cells. Moreover, in both normal and hyperplastic SSE, CK10 expressed strongly in 100% of squamous cells, while it was negative in poorly-differentiated SCC. In addition, 60.5% and 14% of squamous cells in dysplastic epithelium and well-differentiated SCC expressed CK10, respectively [Figure 1]. Results of this study related to the expression of CK10 showed significant statistical differences ($P < 0.01$) [Table 1 and Figure 2]. These results might indicate that the disappearance of keratin 10, which might suggest the early changes of malignant transformation in the squamous cells. Similar results were reported by Kannan *et al*.,[28] who found that keratin 10 being paired with keratin 1 (CK1/10) has potential prognostic value in premalignant oral lesions.

According to the previous results, it can be suggested that the disappearance of CK10 in dysplastic epithelium can be considered as the first stage in the malignant transformation of the SSE.

In this study, the expressions of CKs 18 and 19 (expressed with CKs 18-dc10 and 19-abs10) were confined to the basal cells in normal epithelium, while 7% and 9.8% of squamous cells expressed CKs 18 and 19 in poorly-differentiated SCC, respectively ($P = 0.001$). These results might indicate that the presence of keratins 18 and 19, which might suggest early changes of malignant transformation in the squamous cells. Fillies *et al*.[25] also reported that oral leukoplakias are advisable to be resected when it show positive expression of CK8/18 or 19. Results of this study showed that the CK18 expression has comparable results to the expression of CK19, similar findings were reported in another study.[25,29]

In view to these results, the disappearance of protein filaments 10 may be related to an injury caused by the carcinogenic factors that lead to the breakdown of CK10 and replacing it with CKs 18 and 19. Other theory might be that CK18 and CK19 are the immature forms of CK10. Thus, the quick dysplastic and malignant changes of squamous cells do not permit enough time for maturation; therefore, it remains in the squamous cells in the form of keratin 18 or 19. This hypothesis is in agreement with the study of Santos *et al*.,[30] who found a possible correlation between the presence of CK10 and delay in tumor development.

Despite that Yoshida *et al*.[31] indicated that reduced expression of CK14 might be considered as an indicator for potential malignant transformation. However, the results of this study did not show any differences in the expression of CK14 and CK16 among diverse groups. Expression of CK14 was strongly positive in all squamous cells among all groups while expression of CK16-ab1 was mild positive/negative in all squamous cells among all groups. These results confirm that CK14 and CK16-ab1 have no prognostic value in oral premalignant lesions.

CONCLUSION

Early atypical changes of OSCC may begin on the skeleton of the squamous cells. The gradual disappearance of CK10-ab1 was detected to be normal throughout atypical epithelia. Therefore, CK10 can be considered as a predictable marker for early detection of OSCC. CKs 18-dc10 and 19-abs10 increased gradually at the same time. Such changes in the protein mapping of squamous cells need more investigation for a better understanding of OSCC.

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Conflicts of interest

There are no conflicts of interest.

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