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The Diagnostic Significance of Cytokeratin 13 & 17 Expression in Oral Mucosal Biopsies by Immunohistochemical Technique

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Author`s Contribution

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ABSTRACT

Objective: To find out immunohistochemical (IHC) profile of cytokeratin 13 and 17 expression in oral premalignant and malignant epithelial lesions.

Methodology: This descriptive cross-sectional study was conducted at, pathology department of Liaquat University of medical and health science Jamshoro from January 2017 to June 2019. Excisional biopsy samples of patients of all age groups, both gender with complain of oral mucosal epithelial lesions were enrolled. Oral mucosal biopsies were fixed in 10% formalin, routinely processed, and stained with H&E for histopathological diagnosis. Immunohistochemistry for Cytokeratin 13 and 17 was performed on paraffin sections after deparaffinization and blocking of endogenous peroxidase activity. Tissue sections were kept moist throughout the procedure and mounted on FLEX IHC slides. All the relevant pathological and clinical data were recorded using study proforma and analyzed by SPSS version 20.

Results: Patient's mean age was 40.45±11.94 years and males were in majority 63.4%. Buccal mucosa, cheek and tongue were most common sites of biopsy, 32.8%, 21.6% and 31.3% respectively. CK13 was positive among 73 cases. CK17 was positive among 96 cases. Leukoplakia, sub mucous fibrosis, ulcers were significantly associated with CK13 p=0.003, while leukoplakia and squamous cell carcinoma were significantly associated with CK 17 p=0.001.

Conclusion: The CK13 concluded to be the reliable marker of dysplasia and CK 17 revealed as the best diagnostic marker of SCC. CK13 was significantly higher in leukoplakia, sub mucous fibrosis, ulcers and epithelial dysplasia, while CK17 was higher among cases of leukoplakia and SCC.

Keywords: Oral mucosa, epithelial lesions, CK 13, CK 17.

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Introduction

The oral carcinoma, predominantly oral squamous cell carcinoma (OSCC) remains a significant health challenge throughout the world,[1] due to its higher occurrence and frequently delayed diagnosis, specifically in areas where tobacco and betel nut uses are highly prevalent. It is a most important form of oral carcinoma, comprising around 80% to 90% of all malignancies of the oral cavity, with its incidence varies based on demographics and geographic locations of the population.[2] The early diagnosis of premalignant lesions and accurate differentiation between benign, dysplastic, and the malignant states are very important to improve prognosis and the survival rate. Though histopathological assessment of hematoxylin and eosin stained sections is the standard of diagnosis, this method reveals the inter-observer inconsistency and inadequate sensitivity for early neoplastic alterations. Subsequently, immunohistochemical biomarkers which reflects the specific molecular modifications have been identified to improve the diagnostic precision in the oral epithelial lesions.[3]

The Cytokeratins form a family of the intermediate filament proteins which are key structural factors of epithelial cells and show different expression profiles corresponding to type of cell and status of the differentiations. Such patterns of the expression are changed during dysplasia and carcinogenesis stage, producing CKs as helpful IHC markers in the pathological diagnosis. The CK13 and CK17 are two such cytokeratins with mutual pattern of expression in oral epithelial alteration.[4,5] The CK13 is usually expressed in the supra-basal layers of the normal non-keratinized and the oral epithelium, indicating differentiation of the normal epithelium. On the other hand, CK17 is typically absent in the normal oral mucosa however becomes expressed in the proliferating and activated or the epithelia and malignant transformation perspectives.[5]

Several studies have stated that loss of the CK13 expression associates with transformation of dysplasia and malignancy of oral mucosa.[6] CK13, being a difference marker, demonstrations strong and diffuse staining in the normal epithelial lesion, while the expression of it markedly decreases as the architecture epithelium becomes dysplastic or the neoplastic.[6] Such pattern reflects the loss of normal differentiation and documented in the studies dysplasia and OSCC of the of oral epithelium.[7] On the other hand, expression of CK17 is upregulated during the progression of dysplasia and in OSCC, indicating involvement in the activation of epithelium and carcinogenic progressions. The expression of CK17 increases progressively from normal oral mucosa over dysplasia to invasive carcinoma, considering it a good marker for early evaluation and classifying of lesions of malignancy.[8]

However, in the population from Pakistan, the most of the oral malignant cases are distinguished at advanced phases and the delayed diagnosis is linked to the substantially higher expenses of the treatment, very limited affordability, suboptimal outcomes of therapy and raised rates of mortality.[6] Although IHC application to evaluate the CK13 and CK17 in the routine specimens of the biopsies offers useful advantages as formalin-fixed, paraffin-embedded tissues are available widely available and the techniques of IHC are identical in the most laboratories of pathology. To evaluate the diagnostic effectiveness of CK13 and CK17 expression may possibly therefore enhance the conventional histopathology, serving clinicians to more correctly detect the early dysplastic alterations and differentiation between dysplasia and the OSCC.[3,9] Additionally, quantitative or semiquantitative evaluation of these markers may increase reproducibility and afford objective measures to support decision-making clinically. After assuming the worldwide burden of OSCC and the clinical challenges specifically at local level in the early detection and perfect histopathological differentiation, additional studies into the diagnostic effectiveness of CK13 and CK17 IHC expression in the biopsies of oral mucosa are needed,[9,10] to the potential refine of diagnostic procedures and ultimately progress the outcomes of patients. Hence present study has been conducted Evaluate the effectiveness of IHC profile of cytokeratin 13 and 17 expression in oral premalignant and malignant epithelial lesions.

Methodology

Present descriptive cross-sectional study was conducted at, pathology department of Liaquat University of medical and health science Jamshoro and Diagnostic and research laboratory Hyderabad from January 2017 to June 2019. The sample calculation was done using the open Epi software for sample size calculation Assuming prevalence of oral squamous cell carcinoma as 10%. The estimated sample size was 139 cases at using the 95% confidence interval and 5% of margin of error. Conventional sampling/non-probability technique was used. All the excisional biopsies of patients of all age groups, both genders with complain of oral mucosal epithelial lesions.ie white lesion, ulcer, erythroplakia were included. Whereas known cases of oral malignant lesions, specimen receives without formalin were excluded. The study was performed after the permission of ethical committee of hospital and written informed consent for the study and procedure was obtained from the patient or next of kin. All oral mucosal biopsies, fixed in 10% formalin and after standardized processing, slides were prepared, stained by Hematoxylin and Eosin staining. And initial histopathological diagnosis was made.

Immunohistochemistry: Serial sections of 3-4 mm from the paraffin blocks, Cytokeratin 13 and 17 were used on 4-µm-thick TMA sections. After deparaffinization and rehydration, endogenous peroxidase activity was blocked for 30 min in methanol containing 0.3% hydrogen peroxide. All formalin fixed received specimen were processed for H&E staining and histopathological diagnose confirm.

Immunohistochemical Procedure; Cytokeratin 17 (CK17)

Formalin-fixed, paraffin-embedded tissue specimens were sectioned at approximately 4 µm thickness. Sections were

mounted on FLEX IHC microscope slides to enhance tissue adherence. Heat-induced epitope retrieval (HIER) was performed using the Dako PT Link system with EnVision FLEX Target Retrieval Solution, High pH (50×, code K8004), following the manufacturer's instructions. After pretreatment, tissue sections were incubated with the primary anti-CK17 antibody. Detection was performed using the EnVision FLEX detection system. Following staining, sections were dehydrated, cleared, and permanently mounted. Throughout the procedure, care was taken to prevent tissue drying at any step.

Cytokeratin 13 (CK13)

For CK13, paraffin-embedded, formalin-fixed tissue sections were subjected to HIER using 10 mmol/L Tris buffer with 1 mmol/L EDTA at pH 9.0, which provided optimal antigen retrieval. Other retrieval solutions, including citrate buffer (pH 6.0) and Dako Target Retrieval Solutions (codes S1700 and S3308), were found less effective and were not used. Proteinase K treatment was omitted. Sections were then incubated with the anti-CK13 primary antibody and processed using the EnVision FLEX detection system. Positive controls were included in every run to ensure staining reliability; any run in which the positive control failed to demonstrate staining was considered invalid.

All staining steps were performed in a humidified chamber to prevent tissue desiccation. After completion of the IHC procedure, slides were examined under a light microscope, and staining intensity and distribution were recorded for subsequent analysis. All the data was entered in excel and converted into SPSS version 20 for purpose of analysis.

Results

Patients mean age was 40.45±11.94 years and males were in majority as 63.4% compared to females 36.6%. Leukoplakia was most common lesion 22.3%, Erythroplakia 10.1% cases, epithelial dysplasia 5.0% and ulcer 2.2%. Table:1.

Table I: Demographic statistics of study participants. (n=139)

Demographic variables	Frequency	Percentage
Gender		
Male	88	63.3%
Female	51	36.7%
Type of oral lesions		
Visible growth	82	59.0%
Leukoplakia	35	25.2%
Erythroplakia	17	12.3%
Ulcer	03	2.2%
Submucous Fibrosis	02	1.4%

Well differentiated SCC was found in 54.3% cases, followed by moderate differentiated SCC 34.5% and poorly differentiated SCC 11.2% out of 116 patients, while dysplasia was in remaining 14 cases. Figure 1.

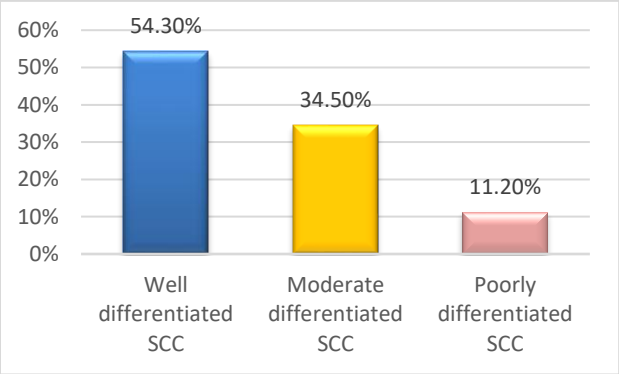

Figure 1. Types of SCC. (n=116)

According to the reactivity, for CK13 the weak reactivity was noted in 25.9% cases, moderate and strong CK13 reactivity were less frequent in 11.5% and 15.1% of cases while around half of the samples showed no reactivity (47.5%). On the other hand, CK17 showed strong reactivity in 12.9% specimens and moderate reactivity in 25.2% cases with weak and negative reactivity each present in about one-third of the specimens (30.9%). Table II.

Table II: Frequency of reactivity of CK13 and CK 17. (n=134)

Variables	Frequency	Percentage
CK13		
Strong Reactivity	21	15.1%
Moderate Reactivity	16	11.5%
Week Reactivity	36	25.9%
Negative	66	47.5%
Total	139	100.0%
CK17		
Strong Reactivity	18	12.9%
Moderate Reactivity	35	25.2%
Week Reactivity	43	30.9%
Negative	43	30.9%
Total	139	100.0%

In this series there was no significant difference in CK 13 and CK 17 according to gender; p-values were quite insignificant. Table III

Table III: CK13 and CK 17 according to gender. (n=134)

Variables	Gender		P-Value
	Male (n=85)	Female (n=49)	
CK13			
Positive	51	22	**0.143**
Negative	37	29	
CK17			
Positive	57	39	**0.150**
Negative	31	12	

According to histological lesion dysplasia was found significantly associated with positive CK13 and squamous cell carcinoma was significantly associated with CK17 p-value<0.05 as shown in table IV.

Table IV: SCC according to CK13 and CK 17. (n=116)

Variables	SCC			
	Wd SCC	Md SCC	Pd SCC	P-Value
CK 13				
Positive	15	19	03	0.076
Negative	48	21	10	
CK 17				
Positive	50	28	05	0.004
Negative	13	12	08	

Wd SCC = Well differentiated SCC, Md SCC = Moderate differentiated SCC, Pd SCC = Poorly differentiated SCC

Discussion

The OSCC considerer as the most prevailing oral cancer, representing a significant global healthcare burden, with a high number of lip and mouth cancers, higher mortality rate and lower 5 years survival rate mostly occurring due to late diagnosis.[11] This study assessed the immunohistochemical profile of cytokeratin 13 and 17 expression in oral premalignant and malignant epithelial lesions among total 139 cases. The mean age of patients was 40.45±11.94 years and majority of males 63.4% compared to females (36.6%), which aligning a study conducted by Matsuhira et al[12] reported relatively higher mean age 61.5 years, with male gender predominance (58.3%), suggesting similar pattern of more common carcinoma presentation among patients with growing age and male gender. Similar findings were documented in the studies conducted by Kiani et al.[13] stating 59.4% males and 60.63 years of mean age, and Irani et al[14] also showed general trend of the carcinoma in older patients and male gender.

In this cohort, the leukoplakia was most common around 22.3%, erythroplakia 10.1%, epithelial dysplasia 5.0% and ulcer 2.2%. Consistent patterns of findings were reported in the study conducted by de Azevedo et al.[15] where Leukoplakias (74.6%) was the most frequent presentation, while erythroplakias (0.8%) represented less frequently compared to our findings and additionally they reported more frequent mild dysplasia cases (33.5%) than our subjects. Consistently, Lapthanasupkul et al[16] documented a high frequency of leukoplakia (1.7%), erythroplakia (0.13%).

In current study, well differentiated SCC was predominantly found in 54.3% cases, followed by moderate differentiated SCC in 34.5% cases, and poorly differentiated SCC in 11.2% out of 116 patients, 14 cases of dysplasia were also in these cases. In agreement with our findings, in the study of Singh et al.,[17] histological grading showed well-differentiated OSCC (43.2%) as the most common carcinoma, however poorly differentiated (23.9%) more frequent than moderately differentiated tumors (23.2%). They further reported epithelial dysplasia among 27.5% of oral lesions. Similarly, a study conducted by Ghatage et al.[18] claimed well-differentiated carcinoma (64.8%) as the most frequent grade, followed by moderately differentiated (13.9%) and poorly differentiated carcinoma (2.4%).

In this series there was no significant difference in CK 13 and CK 17 according to gender; p-values were quite insignificant. Comparable to these findings, Tojyo et al[19] found no significant difference in the expression of CK 17 according to gender. Correspondingly, in the study of Chang and Wang et al[20] expression of CK13 and CK17 showed no significant association with gender. These findings suggest that there is no influence of gender on the expression of CK 13 and CK 17 in OSCC.

In our study, according to histological lesion, dysplasia was found significantly associated with positive CK13 and squamous cell carcinoma was significantly associated with CK17 (p= <0.05). In line with our findings, in the study conducted by Kitamura et al[21] revealed statistically significant expression of CK17 in well-differentiated carcinoma and in dysplastic leukoplakias (p =0.01), indicating association of CK17 with malignant lesions. On the other hand, expression of CK13 was significant in hyperplastic leukoplakia (p < 0.01), suggesting significant changes in CK13 expression with dysplasia regulation. Consistent findings were documented by Sanguansin et al[8] where in oral carcinoma, CK17 was overexpressed and significantly associated with histopathologic OSCC grading and dysplasia, suggesting pivotal role of CK17 in oral carcinoma development, making it a valuable diagnostic marker. The findings of the studies including this one supports the diagnostic value of CK13 and CK17 in oral epithelial lesions and OSCC; though, certain limitations like relatively limited sample size of the study may restrict the reliability of the findings. Few variations across different studies may be due to variations in sample size of studies, characteristics of population, types and stages of the lesions, immunohistochemical techniques and the scoring criteria for the expression cytokeratin. Hence further additional large-scale research should focus on multicenter studies with long-standing follow-up to validate the observations and to explore the significance of prognosis of CK13 and CK17, separately or in

combination with the other markers, in the early evaluation and progression of oral disorders of malignancy.

Conclusion

The CK17 revealed as a more valuable diagnostic marker for SCC, as its expression increases with transformation of malignancy, with higher expression specifically in leukoplakia and SCC, indicating its role in identifying epithelial activation and malignancy, while CK13 observed as a best marker of dysplasia, as its loss was positively correlated with severity of dysplasia and it was significantly higher in leukoplakia, sub mucous fibrosis, ulcers and epithelial dysplasia. Future additional relevant large-scale studies are recommended to validate the findings.

References

1. Ramachandran S. Oral cancer: recent breakthroughs in pathology and therapeutic approaches. Oral Oncol Rep. 2024;12:100678. doi:10.1016/j.oor.2024.100678
2. Badwelan M, Muaddi H, Ahmed A, Lee KT, Tran SD. Oral squamous cell carcinoma and concomitant primary tumors: what do we know? A review of the literature. Curr Oncol.2023;30(4):3721-3734. doi:10.3390/curroncol30040283
3. Gyanchandani A, Shukla S, Vagha S, Acharya S, Kadu RP. Diagnostic utility of cytokeratin 17 expression in oral squamous cell carcinoma: a review. Cureus. 2022;14(7):e27041. doi:10.7759/cureus.27041
4. Nobusawa A, Sano T, Negishi A, Yokoo S, Oyama T. Immunohistochemical staining patterns of cytokeratins 13, 14, and 17 in oral epithelial dysplasia including orthokeratotic dysplasia. Pathol Int. 2014;64(1):20-27. doi:10.1111/pin.12125
5. 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):1299-1310. doi:10.1007/s00432-012-1202-6
6. Farrukh S, Syed S, Pervez S. Differential expression of cytokeratin 13 in non-neoplastic, dysplastic and neoplastic oral mucosa in a high-risk Pakistani population. Asian Pac J Cancer Prev. 2015;16(13):5489-5492. doi:10.7314/APJCP.2015.16.13.5489
7. Lennartz M, Ullmann VS, Gorbokon N, Uhlig R, Rico SD, Kind S, et al. Cytokeratin 13 (CK13) expression in cancer: a tissue microarray study on 10,439 tumors. APMIS. 2023;131(2):77-91. doi:10.1111/apm.13280
8. Sanguansin S, Kosanwat T, Juengsomjit R, Poomsawat S. Diagnostic value of cytokeratin 17 during oral carcinogenesis: an immunohistochemical study. Int J Dent. 2021;2021:4089549. doi:10.1155/2021/4089549
9. Kiani MN, Asif M, Ansari FM, Ara N, Ishaque M, Khan AR. Diagnostic utility of cytokeratin 13 and cytokeratin 17 in oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Biol. 2020;5(4):153-158. doi:10.31557/apjcb.2020.5.4.153-158
10. Pan CH. Targeting keratin 17-mediated metabolic rewiring overcomes chemoresistance and uncovers therapeutic opportunities for pancreatic cancer [dissertation]. Stony Brook (NY): State University of New York at Stony Brook; Year unknown.
11. Parlatescu I, Serban AE, Tovaru S, Perlea P. Diagnostic and prognostic biomarkers in oral leukoplakia and oral squamous cell carcinoma: a narrative review. Oncologie. 2026;12:Article number pending. doi:10.1515/oncologie-2025-0429
12. Matsuhira A, Noguchi S, Sato K, Tanaka Y, Yamamoto G, Mishima K, et al. Cytokeratin 13, cytokeratin 17, Ki-67 and p53 expression in upper layers of epithelial dysplasia surrounding tongue squamous cell carcinoma. Bull Tokyo Dent Coll. 2015;56(4):223-231. doi:10.2209/tdcpublication.56.223
13. Irani S. Pre-cancerous lesions in the oral and maxillofacial region: a literature review with special focus on etiopathogenesis. Iran J Pathol. 2016;11(4):303-322.
14. de Azevedo AB, Dos Santos TC, Lopes MA, Pires FR. Oral leukoplakia, leukoerythroplakia, erythroplakia and actinic cheilitis: analysis of 953 patients focusing on oral epithelial dysplasia. J Oral Pathol Med. 2021;50(8):829-840. doi:10.1111/jop.13183
15. Lapthanasupkul P, Poomsawat S, Punyasingh J. A clinicopathologic study of oral leukoplakia and erythroplakia in a Thai population. Quintessence Int. 2007;38(8):e448-e455.
16. Singh S, Singh J, Chandra S, Samadi FM. Prevalence of oral cancer and oral epithelial dysplasia among North Indian population: a retrospective institutional study. J Oral Maxillofac Pathol. 2020;24(1):87-92. doi:10.4103/jomfp.JOMFP_347_19
17. Ghatage DD, Kendre AG, Palve DH, Dhobley A, Ghodichor D, Lakawath A. Oral squamous cell carcinoma: a 26 years institutional cross-sectional study. J Oral Maxillofac Pathol. 2024;28(2):240-246. doi:10.4103/jomfp.jomfp_38_24
18. Tojyo I, Shintani Y, Nakanishi T, Okamoto K, Hiraishi Y, Fujita S, et al. PD-L1 expression correlated with p53 expression in oral squamous cell carcinoma. Maxillofac Plast Reconstr Surg. 2019;41(1):56. doi:10.1186/s40902-019-0239-8
19. Chang JY, Wang YP. The correlation of p53, CK13, CK17, and Ki67 immunostaining patterns in assisting the histopathologic grading of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol. 2024;138(2):e53. doi:10.1016/j.oooo.2024.04.070