International Journal of Biomedicine 12(4) (2022) 596-600
http://dx.doi.org/10.21103/Article12(4)_OA13

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Assessment of the Relationship between Expressions of CD34, p63 with Different Clinical Types of Oral Epithelial Dysplasia: A Retrospective Immunohistochemical Study

Mustafa Mohammed Abdulhussain[1*], Fawaz D. Alaswad[2]

[1]*Department of Oral Pathology, College of Dentistry, Mustansiriyah University, Baghdad, Iraq*
[2]*Department of Oral Diagnosis, College of Dentistry, Baghdad University, Baghdad, Iraq*

Abstract

Background: Potentially malignant disorders such as leukoplakia and erythroplakia are often associated with dysplastic changes that have an increased risk for malignant transformation. CD34 is considered as an important marker for tissue vascularization, which represents microvessel density. P63 has a role in epithelial proliferation and is frequently altered in dysplasia and associated with tumorigenesis. The aim of this study was to evaluate the expression of CD34 and p63 in different grades of oral epithelial dysplasia (OED).

Methods and Results: This research included 50 histopathologically confirmed OED. Grading classification of OED was determined according to the WHO criteria, in which the lesions were classified into mild, moderate, and severe grades. CD34 and P63 expressions were studied by using the immunohistochemical technique. Most OED lesions were observed in patient between 40 and 69 years of age. Buccal mucosa was the most affected site (42%). According to histopathological grades, mild OED was predominant (54%). There was a significant difference among OED grades through the P63 marker.

Conclusion: The P63 marker can be considered a good indicator for malignant transformation by grade scoring scales, and the CD34 marker can be used as a useful diagnostic indicator for OED**. (International Journal of Biomedicine. 2022;12(4):596-600.**).

Keywords: oral epithelial dysplasia • malignancy • CD34 • P63 • immunohistochemistry

For citation: Abdulhussain MM, Alaswad FD. Assessment of the Relationship between Expressions of CD34, p63 with Different Clinical Types of Oral Epithelial Dysplasia: A Retrospective Immunohistochemical Study. International Journal of Biomedicine. 2022;12(4):596-600. doi:10.21103/Article12(4)_OA13.

Abbreviations

AUC, area under the curve; **MVD**, microvessel density; **OED,** oral epithelial dysplasia; **OSCC**, oral squamous cell carcinoma; **OPMDs**, oral potentially malignant disorders.

Introduction

Oral epithelial dysplasia (OED) is characterized by a spectrum of histologic changes in the oral mucosa with the potential to transform into oral squamous cell carcinoma (OSCC).[1,2] A meta-analysis of OED data indicates a malignant transformation rate of 12% within 2 years, increasing to 22% within 5 years.[3] Predicting the risk of malignant transformation is predominantly based on clinicopathologic correlation, histologic examination, and grading.[2,3] However, there is currently no consensus regarding the risk of malignant transformation based on histopathology.[5]

Oral potentially malignant disorders (OPMDs) are defined as a group of oral mucosal lesions (leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus, oral dysplasia),[6,7] which are associated with an increased risk of malignant transformation. While OPMD is a clinical term, OED is a histo-morphologically spectrum of epithelial changes associated with an increased risk of transformation to carcinoma.[8] Many OPMD may be pathologically associated with OED.[9-11] The OED histological criteria reported in the WHO classification 2017 are based on “architectural” (disordered tissue organization) and “cytological” (individual cell abnormality) changes.[12-14] However, the malignant

potential of OED can be variable and unpredictable. OSCC has been shown to develop in the absence of known OED and without any evidence of neoplasia seen histologically in previously and conventionally stained oral biopsies in >20% of previously biopsied cases.[15] However, the overall evidence indicates a positive correlation between the likelihood and time of malignant transformation with increasing degrees of dysplasia. [9,11,14]

CD34 is a glycoprotein member found on the surface of a number of body cells. It is a group of differentiation compounds that act as cell adhesion proteins.[16] During the formation of a tumor, angiogenesis is the complicated creation of newly created blood vessels from pre-existing vascular complexes through vessel expansion.[17] In epithelial dysplastic lesions, angiogenesis is believed to be critical for the nourishment and proliferation of dysplastic cells as well as tumor cells, mesenchymal cells, and inflammatory cells like mast cells and macrophages secreting angiogenic materials. Amplified levels of angiogenic triggers like vascular epidermal growth factor (VEGF) result in angiogenesis.[18]

In many tumors, endothelial cells of blood vessels play an essential role in angiogenesis, and CD34 is a signal for these cells. Microvessel density (MVD) in different malignancies is evaluated using the CD34 marker. Furthermore, MVD helps in predicting tumor progression or regression.[19]

The p63 is essential for regulating epithelial cell proliferation, development, and maturation, and its level in the epithelial dysplastic lesions is usually changed.[20] The p63 is implicated in embryogenesis, cell differentiation, and defective cell death.[21] It is also involved in the formation of stratified squamous epithelium.[22] Its role in the oral epithelium might be to preserve stem cell activity rather than a direct link between tumorigenesis and metastasis transformation.[23]

The purpose of this research was to evaluate the expression of CD34 and p63 in different grades of OED.

Materials and Methods

Fifty cases of OED were retrieved from the archives of the Oral Pathology Laboratory of the Oral Diagnosis Department at the College of Dentistry (Baghdad University). Formalin-fixed paraffin-embedded (FFPE) histopathologically diagnosed tissue specimens were collected along with their relevant patient's clinical data (age, sex, and site) as provided from the oral and maxillofacial reports.

Grading classification was determined according to the WHO criteria, in which the lesions were classified into mild, moderate, and severe grades.[24] Hematoxylin and eosin (H&E) stained tissue sections were evaluated by two pathologists to confirm the diagnosis. The positive tissue controls were the human tonsil for CD34 and the human prostate for P63.

The presence of a brown granular DAB dye pattern within the particular cellular or tissue compartment for a specific antibody in positive control tissue slides, according to the product's datasheets, and the lack of staining in negative control tissue samples indicated immunohistochemical signal specificity. The markers' expression was quantitatively assessed.

CD34 and microvessel density quantification

The immunohistochemical expression of CD34 was assessed to determine microvessel density (MVD), which represents the number of microvessels in each field at 40 magnifications, and the average was calculated. Individual endothelial cells or clusters of endothelial cells with or without a lumen that established a brown color with anti-CD34 were regarded as positively stained blood vessels.[25]

P63 scoring

The positive nuclear staining for P63 was assessed using a well-established quantitative scoring system ranging from negative to strong positive staining, as follows:[26]

- Negative staining (less than 5% stained cells)
- Weak positive staining (between 5% and 25% stained cells)
- Moderate positive staining (between 25% and 50% stained cells)
- Strong positive staining (more than 50% stained cells)

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Baseline characteristics were summarized as frequencies and percentages. Group comparisons with respect to categorical variables are performed using One-Sample Chi-Square test. A probability value of $P<0.05$ was considered statistically significant.

Results

The distribution of socio-demographic characteristics such as age groups and gender, as well as comparison significance, were demonstrated to be sure whether these two variables regarding studied patients have randomly distributed among their different classes or not.

The age group's distribution of OED patients showed no significant difference ($P>0.05$). Thus, the probability of OED did not differ according to the age groups, as well as age pivoted in the fifth and sixth decades, with mean value and standard deviation (53.36±12.63 years). As for gender, there was no significant difference ($P>0.05$), and the probability of OED does not differ according to the gender's patients (Table 1).

Table 1.

Demographic characteristics of OED patients.

Variable		n	%	Statistics*
Age group (years)	< 40	7	14	χ^2= 5.600 P=0.231
	40 _ 49	12	24	
	50 _ 59	13	26	
	60 _ 69	13	26	
	≥ 70	5	10	
	Mean ± SD 53.36 ± 12.63			
Gender	Male	24	48	P=0.888
	Female	26	52	
(Between Age & Gender)		CC = 0.334 P = 0.178		

**Testing based on One-Sample Chi-Square test, Binomial test, and Contingency Coefficient (CC) measuring test.*

The distribution of the site and grade of OED, as well as comparison significance, are present in Table 2.

Table 2.

Site and Grade distributions of OED in study patients

Variable		n	%	Statistics*
Site	Tongue	12	24	χ^2= 8.720 *P*=0.033
	Buccal Mucosa	21	42	
	Lips	10	20	
	Others	7	14	
Grade	Mild	27	54	χ^2= 13.240 *P*=0.001
	Moderate	17	34	
	Sever	6	12	
(Site & Grade)		CC = 0.318 *P* = 0.469		

**Testing based on a One-Sample Chi-Square test and Contingency Coefficient (CC) measuring test.*

Buccal mucosa was the most affected site (42%), followed by the tongue site (24%), lips site (24%), and other sites (14%).

According to histopathological grades (Figure 1), a mild grade of OED was predominant (54%), followed by a moderate grade (34%) and a severe grade (12%) (Table 2).

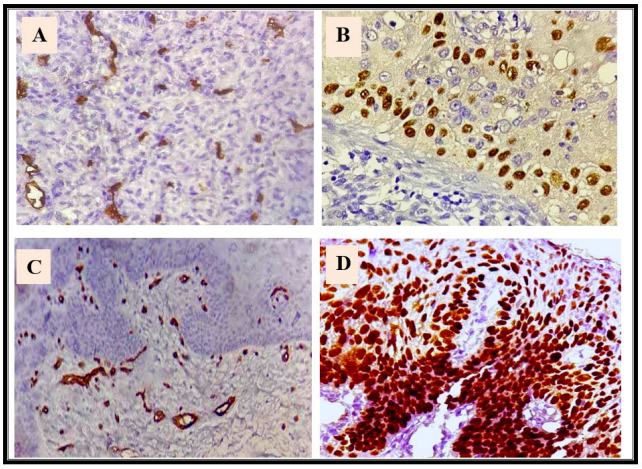

Fig. 1. *A - Photomicrograph of the positive controls of CD34 reactivity in human tonsil, B - Photomicrograph of the positive controls of P63 reactivity in human prostate, C - Positive membranous immunohistochemical expression of CD34 in OED (mild grade), D - Positive nuclear immunohistochemical expression of P63 in OED (severe grade), (magnification 20X).*

Despite there being no significant AUC by the CD34 marker (*P*>0.05) under the guideline of the lowest grade group, which was adopted as the control group, analysis for the compromised pairs (Grade III as a target group and Grade I as a baseline group and Grade III as a target group and Grade II as a baseline group) showed that CD34 marker could be more than an extended indicator for diagnosing OED and especially for the last combination since the AUC decreased by more than half, which confirms a decrease in the CD34 marker with the severe grade of OED (Figure 2).

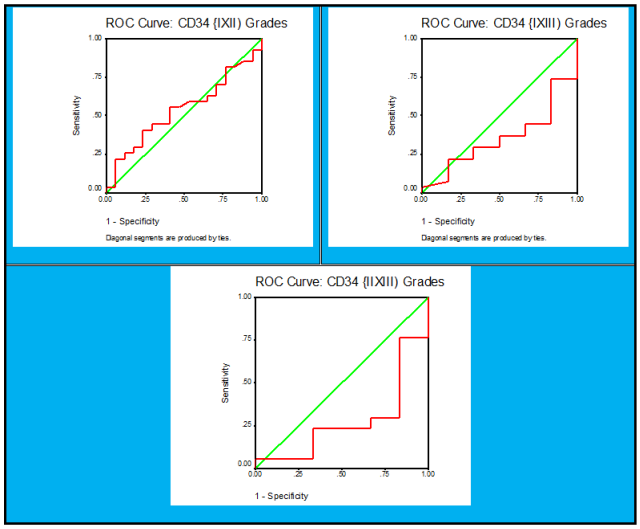

Fig. 2. *ROC-curve plots for the CD34 marker for the group grades with different probable combinations*

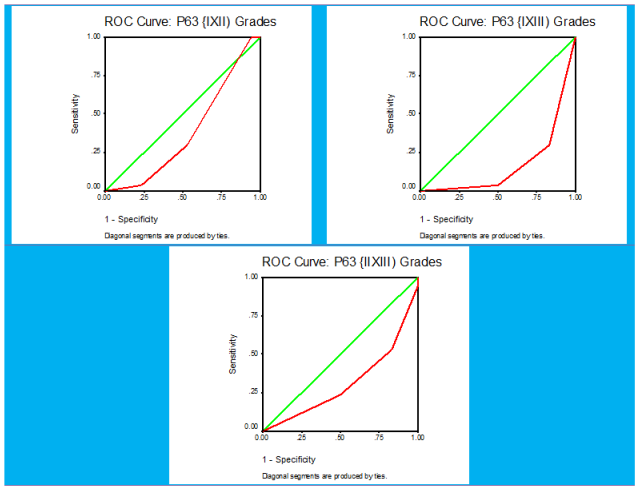

Fig. 3. *ROC-curve plots for the p63 marker for the group grades with different probable combinations*

The significant AUC by the P63 marker (*P*<0.05) under the guideline of the lowest grade group, which was adopted as the control group, and that was accounted at the compromised pair (Grade III as a target group and Grade I as a baseline group) showed that the P63 marker could be a very good indicator for predicting the malignant transformation of OED. AUC could be close to the estimated value (0.000) with a study pair, as indicated by the 95% CI (Fig. 3).

Discussion

Histopathological assessed severity of OED remains essential for the prediction of malignant transformation of precancerous lesions.[27] In this study, most of the OED cases were distributed in the fifth and sixth decades of life (mean age of 53.36±12.63 years), which was consistent with the results of another study.[28] This similarity might be due to a similar sample size and gender distribution. The association of OED development

with aging could be explained by the prolonged accumulation of genetic changes caused by genetic and/or environmental factors such as tobacco and alcohol drinking, which are considered provoking factors in epithelial dysplasia development.

Concerning gender distribution, the results of the current study showed that females were slightly more affected by OED (52%) than males (48%). Similar findings were reported in other parts of the world.[29] Batool et al.,[30] in contrast to our study, showed that 80% of oral dysplastic samples belonged to males with the M/F ratio of 4:1. There is controversy as to which gender is most affected. Specific habits, such as betel nut or tobacco chewing, could explain this in each region.

According to the site lesions, the distribution of studied cases showed that the buccal mucosa was the commonest affected site with OED (42%). This finding was in agreement with the previous study.[30] The higher prevalence of the buccal mucosa site is probably related to the widespread habit of betel/areca nut chewing in some regions and increased malignant transformation in oral leukoplakia lesions of the buccal mucosa over other sites. Napier et al.[31] found that OSCC was more likely with potentially malignant disorders on the lateral and ventral tongue, the floor of the mouth, and retromolar/soft palate complex than those elsewhere.

In our study, the distribution of OED grades (mild, moderate, and severe) was characterized by significant differences. Grading of OED continues to be a hotly debated subject due to subjectivity. Moreover, several grading systems are currently employed because of the need for a consensus. It should be recognized that OED grade is of limited utility as a risk prediction marker for OSCC. [15,30]

The high score of the CD34 marker was found in the severe grade of OED cases, followed in descending order by the mild grade and, finally, moderate grade, so no statistical significance was obtained among the grades of OED for CD34 expression. This finding is in concordance with previous studies,[31] which stated that the confirmed theory that the increased nutrient requirements of actively developing and dividing cells lead to an increase in angiogenesis, which in turn leads to tumor growth.

Pujari et al.[32] showed a strong statistical significance in MVA with the grade of dysplasia when mild and moderate epithelial dysplasia were compared to severe epithelial dysplasia. Thus, the increased MVA might be related to numerous cytokines, macrophages, mast cells, and neutrophils triggered by the dysplastic epithelium's altered keratinocytes. The number of dysplastic cells that can create chemical signals that can start the angiogenesis process grows as the dysplasia degree increases. Thus, angiogenesis promotes tumor growth by supplying nutrients and oxygen. It might be utilized as a prognostic marker to determine the aggressiveness of dysplastic lesions and their transition to carcinoma.[25] The assessment of MVD was done by one study that showed no significant correlation with the dysplastic histological grade.[33] This discrepancy in the results may be due to different sizes in the study sample or subjective assessment of epithelial dysplasia grading.

The results of this study revealed that the vast majority of the score for P63 expression was accounted within a severe grade and suggested that the expression of p63 increased significantly from the mild-to-moderate-to-severe grade of dysplasia. This finding comes in accordance with many other previous studies which showed that the P63 overexpression might be caused by a stabilization of p63 that is not caused by mutations. The rate at which cells turn over a short period of time may also contribute to stabilization by disrupting their breakdown routes or accumulating wild-type proteins.[34] Bavle et al. showed that increased expression of p63 in cases of oral submucous fibrosis can be considered as definitive quantitative markers in the prediction of malignant transformation.[35]

In conclusion, the P63 marker can be considered a good indicator for malignant transformation by grade scoring scales, and the CD34 marker can be used as a useful diagnostic indicator for OED**.**

Competing Interests

The authors declare that they have no competing interests

References

1. Thomas V, Devi SR, Jeyaseelan V, Jeyseelan L. Mucosal Disorders with Oral Epithelial Dysplasia risk–Development of a simple screening tool for general health care setting. Oral Oncol 2012;48(8):671-677.
2. Cheung VKY, Hulme K, Schifter M, Palme C, Low TH, Clark J, Gupta R. Oral Epithelial Dysplasia: A Review of Diagnostic Criteria for Anatomic Pathologists. Adv Anat Pathol. 2022 Jul 1;29(4):227-240. doi: 10.1097/PAP.0000000000000343.
3. Mehanna HM, Rattay T, Smith J, McConkey CC. Treatment and follow-up of oral dysplasia - a systematic review and meta-analysis. Head Neck. 2009 Dec;31(12):1600-9. doi: 10.1002/hed.21131.
4. McCullough MJ, Prasad G, Farah CS. Oral mucosal malignancy and potentially malignant lesions: an update on the epidemiology, risk factors, diagnosis and management. Aust Dent J. 2010;55(suppl 1):61-65. doi.org/10.1111/j.1834-7819.2010.01200.x
5. Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head Neck Pathol. 2007 Sep;1(1):61-6. doi: 10.1007/s12105-007-0014-5.
6. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007 Nov;36(10):575-80. doi: 10.1111/j.1600-0714.2007.00582.x.
7. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jun;125(6):582-590. doi: 10.1016/j.oooo.2018.03.011.
8. Lorini L, Bescós Atín C, Thavaraj S, Müller-Richter U, Alberola Ferranti M, Pamias Romero J, Sáez Barba M, de Pablo García-Cuenca A, Braña García I, Bossi P, Nuciforo P, Simonetti S. Overview of Oral Potentially Malignant Disorders: From Risk Factors to Specific Therapies. Cancers (Basel). 2021 Jul 23;13(15):3696. doi: 10.3390/cancers13153696.
9. Muller S. Oral epithelial dysplasia, atypical verrucous lesions and oral potentially malignant disorders: focus on

histopathology. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:591–602. doi.org/10.1016/j.oooo.2018.02.012
10. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jun;125(6):612-627. doi: 10.1016/j.oooo.2017.12.011.
11. Iocca O, Sollecito TP, Alawi F, et al. Potentially malignant disorders of the oral cavity and oral dysplasia: A systematic review and meta-analysis of malignant transformation rate by subtype. Head Neck. 2020;42:539-555. doi.org/10.1002/hed.26006
12. WHO Classification of Head and Neck Tumours. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, Eds. IARC Press: Lyon, France, 2017.
13. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006 Nov;42(10):987-93. doi: 10.1016/j.oraloncology.2005.12.014.
14. Nankivell P, Williams H, Matthews P, Suortamo S, Snead D, McConkey C, Mehanna H. The binary oral dysplasia grading system: validity testing and suggested improvement. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Jan;115(1):87-94. doi: 10.1016/j.oooo.2012.10.015.
15. Pritzker KPH, Darling MR, Hwang JT, Mock D. Oral Potentially Malignant Disorders (OPMD): What is the clinical utility of dysplasia grade? Expert Rev Mol Diagn. 2021 Mar;21(3):289-298. doi: 10.1080/14737159.2021.1898949.
16. Raffat MA, Hadi NI, Alghamdi O, Al-Aali KA, Al Deeb M, Abduljabbar T, Vohra F. Expression of Salivary S100A7 Levels in Stage I Oral Submucous Fibrosis: A Clinical and Laboratory Study. Asian Pac J Cancer Prev. 2020 Apr 1;21(4):1115-1119. doi: 10.31557/APJCP.2020.21.4.1115.
17. Roskoski R Jr. Vascular endothelial growth factor (VEGF) signaling in tumor progression. Crit Rev Oncol Hematol. 2007 Jun;62(3):179-213. doi: 10.1016/j.critrevonc.2007.01.006.
18. Menakuru SR, Brown NJ, Staton CA, Reed MW. Angiogenesis in pre-malignant conditions. Br J Cancer.2008;99(12):1961-1966. doi:10.1038/sj.bjc.6604733
19. Siar CH, V. P. A. Oo, Nagatsuka H, Nakano K, Ng. KH, Kawakami T. Angiogenic squamous dysplasia-like phenomenon in oral epithelial precursor lesions. Eur J Med Res. 2009;14(7): 315-319. doi.org/10.1186/2047-783X-14-7-315
20. Yong M, Yang L, Suyila Q, et al. Expression and clinical implications of P53, P63, and P73 protein in malignant tumor of the parotid gland. Turk J Med Sci. 2014;44(5):875-882. doi:10.3906/sag-1304-136
21. Yoon MK, Ha JH, Lee MS, Chi SW. Structure and apoptotic function of p73. BMB reports. 2015 48(2):81. doi.org/10.5483%2FBMBRep.2015.48.2.255
22. Mills AA. p63: oncogene or tumor suppressor? Curr Opin Genet Dev. 2006 Feb;16(1):38-44. doi: 10.1016/j.gde.2005.12.001.
23. Takeda T, Sugihara K, Hirayama Y, Hirano M, Tanuma JI, Semba I. Immunohistological evaluation of Ki67, p63, CK19 and p53 expression in oral epithelial dysplasias. J Oral Pathol Med 2006;35(6):369-375. doi.org/10.1111/j.1600-0714.2006.00444.x
24. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization Classification of Tumors. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005.
25. Sundararajan A, Muthusamy R, Gopal Siva K, Harikrishnan P, Kumar SCK, Rathinasamy SK. Correlation of Mast Cell and Angiogenesis in Oral Lichen Planus, Dysplasia (Leukoplakia), and Oral Squamous Cell Carcinoma. Rambam Maimonides Med J 2021;12(2):e0016. doi.org/10.5041%2FRMMJ.10438
26. Kamal KAER, Mohamed MA, Abd-alwahab AS, Alazazi M. P63 & ki67 antigen expression in relation to the aggressiveness of oral squamous cell carcinoma. Egyptian Dental Journal (2-April (Oral Medicine, X-Ray, Oral Biology & Oral Pathology). 2020;(66)1091-1098. doi.org/10.21608/edj.2020.23986.1009
27. Niu LX, Feng ZE, Wang DC, Zhang JY, Sun ZP, Guo CB. Prognostic factors in mandibular gingival squamous cell carcinoma: A 10-year retrospective study. Int J Oral Maxillofac Surg. 2017;46(2):137–143. doi.org/10.1016/j.ijom.2016.09.014
28. Gopinath D, Thannikunnath BV, Neermunda SF. Prevalence of Carcinomatous Foci in Oral Leukoplakia: A Clinicopathologic Study of 546 Indian Samples. J ClinDiagn Res 2016;10:78-83. doi.org/10.7860%2FJCDR%2F2016%2F16815.8305
29. Bijina BR, Ahmed J, Shenoy N, Ongole R, Shenoy S, Baliga S. Detection of human papilloma virus in potentially malignant and malignant lesions of the oral cavity and a study of associated risk factors. South Asian Journal of Cancer. 2016;5(4):179-181. doi.10.4103/2278-330X.195337
30. Batool S, Fahim A, Qureshi A, Anas S, Qamar N, Kamran S. Significance of Expression of Cyclin D as an Early Indicator in Dysplastic Transformation of Oral Mucosa in Tobacco Users. JPDA. 2019;28(4):171-175. https://doi.org/10.25301/JPDA.284.171
31. Napier SS, Speight PM. Natural history of potentially malignant oral lesions & conditions: An overview of the literature. J Oral Pathol Med 2008;37(1):110. doi.org/10.1111/j.1600-0714.2007.00579.x
32. Pujari RV, Vanaki SS, Puranik RS, Desai RS, Motupalli N, Halawar S. Histomorphometric analysis of vascularity in normal buccal mucosa, leukoplakia, and squamous cell carcinoma of buccal mucosa. J Oral Maxillofac Pathol 2013;17:334-339.doi.org/10.4103%2F0973-029X.125178
33. Mohammad DN, Garib BT. CD34 and Wnt3 expression in potentially malignant oral disorders. J Bagh Coll Dent. 2017;29:59-67.
34. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. Blood. 1996 Jan 1;87(1):1-13.
35. Bavle RM, Paremala K, Sowmya M, Sudhakara M, Reshma V, Hosthor S. Predicting the Malignant Transformation of Oral Submucous Fibrosis Using Quantitative Biomarkers p63 and CD31. J Orofac Sci. 2020;12:52-60. doi. 10.4103/jofs.jofs_6_20.

***Corresponding author:** Mustafa Mohammed Abdulhussain, MSc. Department of Oral Pathology, College of Dentistry, Mustansiriyah University. Baghdad, Iraq. E-mail: mustafa80moh@uomustansiriyah.edu.iq*