Immunoexpression of Ki-67 in determining prognosis oral cavity squamous cell carcinoma

Silvi Kintawati[1*], Hening Tjaturina Pramesti[2]

[1]**Department of Oral Biology, Faculty of Dentistry Maranatha Christian University, Indonesia**
[2]**Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia**

ABSTRACT

Introduction: Several researchers have been stated that the right prognosis of oral squamous cell carcinoma (OSCC) can be achieved through assessment of clinical tumour staging and histopathological tumour grading as well. The use of biomarker in histopathological grading is helpful to ensure the prognosis. The objective of this study was to determine the correlation between expression of Ki-67 and histopathological grading of OSCC. **Methods:** A retrospective study was conducted by assessing 20 cases of paraffin-embedded OSCC derived from the Department of Pathology Anatomy of Hasan Sadikin Hospital Bandung in 2013. The OSCC performed was staining by hematoxylin-eosin while antibodies Ki-67 was used in immunohistochemistry reaction to determine the relation of expression of Ki-67 with histopathologic grade of OSCC for predicting prognosis. **Results:** The result shows that distribution of Ki-67 expression varied from 20% to 80% in each case. The ki-67 intensity was also distributed from weak, moderate and strong. **Conclusion:** There is a significant correlation between Ki-67 immunoexpression and the histopathological grading of squamous cell carcinoma ($p < 0.01$); thus, Ki-67 can be used to predict prognosis.

Keywords: Immunoexpression, Ki-67, Oral Squamous Cell Carcinoma (OSCC), prognosis.

p-ISSN 1979-0201; e-ISSN 2549-6212; Available from: http://jurnal.unpad.ac.id/pjd/article/view/22888
DOI: 10.24198/pjd.vol31no2.22888
Submission: Jun 19, 2019; Accepted: Jul 29, 2019; Published online: Jul 31, 2019

INTRODUCTION

Squamous cell carcinoma is a malignancy of the stratified squamous epithelium which can cause local destruction and metastasis.[1,2] Squamous cell carcinoma in oral cavity is the most common tumour in the world and about 90% abnormalities found at the head and neck region in an advanced stage. So, the treatment is more difficult.[3,4,5] The incidence of oral squamous cell carcinoma (OSCC) most often occurs on the lip inferior, lateral and ventral part of the tongue, floor of the mouth, followed by the posterior part of the soft palate, and tonsils area. Moreover, its treatment failure can cause a recurrence of the tumour.[1] Inevitably, the 5-year survival rate will decrease.[4,6]

Clinically OSCC has two types of growth, those are exophytic and endophytic, and their

Corresponding author: Silvi Kintawati, Department of Oral Biology, Faculty of Dentistry Maranatha Christian University, Indonesia. Jalan Surya Sumantri 65, Bandung, West Java, Indonesia 40164. Phone: +62 812-2023-520; Email: silvikintawati@yahoo.com

formation can damage mucosal surfaces typically. Exophytic lesions have an irregular surface and papillomatous, the colour varies from normal to red with white patches depending on the amount of keratin produced. The surface sometimes ulcerated and on palpation of the tumour mass feels hard. On the other hand, endophytic lesion characterised by their curvature, irregular edges, ulceration, borderless elevated (induration) with the red mucosa and white patches.[1] Beside of clinical assessment, oral squamous cell carcinoma can also be diagnosed histopathologically. According to Broders, the degree of carcinoma histopathologically is classified regarding cell proliferation and differentiation. Grade I is well-differentiated, grade II (moderately differentiated), grade III (poorly differentiated) and grade IV (anaplastic).[1]

However, prognosis in OSCC depends on the histopathological grading and clinical staging of the tumour.[1,2,4,7] Rate of dysplasia may also show the following abnormal cell characteristics which include increase in mitotic figures, binucleation and multinucleation, changes in shape and size of nuclear, hyperchromatic, and keratinization.[7] Mild dysplasia, moderate dysplasia to severe dysplasia categories may show abnormalities epithelium with many different grades. The abnormality that involved the entire epithelium thickness can be diagnosed as carcinoma-in-situ. While the damage of the basement membrane and the invasion of the underlying connective tissue can be diagnosed as a carcinoma.[1]

In this study, we used Ki-67 monoclonal antibodies in terms of histopathologic examination of tumour cell proliferation and differentiation. Human cell proliferation is highly believed correlate to the expression of Ki-67 nuclear protein, or it can be used as a potential marker for cell proliferation. Human Ki-67 nuclear protein is expressed in all stage of cell cycles such as G1, S, G2, and mitosis, except for resting stage or G0.2,4,8,9,10 The Ki-67 monoclonal antibodies, will immunoreactive positively to the Ki-67 nuclear protein of tumour cells as antigen.[2,9,10,11,12,13] Prognosis of a tumour is closely related to the biological behaviour of the tumour, the fast and high of proliferation degree of the tumour. The more aggressive tumour, and the worse of prognosis.[9,10,14]

This study was aimed to correlate between the expression of Ki-67 monoclonal antibody and histopathological grading of OSCC in determining the prognosis, identifying therapeutic targets precisely, and avoiding recurrence and metastases.

METHODS

This study has been approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran with the registration number of 0817010023 and approval number of 84/UN6.C1.32/KEPK/PN/2017.

This retrospective study selected and analysed 20 cases of OSCC derived from the formalin fixed-paraffin-embedded tissue of the Department of Pathology, Dr Hasan Sadikin Hospital in 2013. After sectioning, tissue samples were stained with hematoxylin-eosin for histopathologic diagnosis and grading according to Broder's modification. The criteria were respectively, Grade I (Well Differentiated Squamous Cell Carcinoma, WDSCC), Grade II (moderately differentiated Squamous Cell Car-cinoma, MDSCC) and Grade III (poorly differentiated Squamous Cell Carcinoma, PDSCC).[1]

Immunohistochemical staining was also performed on the sectioned tissue samples using a streptavidin-biotin peroxidase technique. Monoclonal antibody Ki-67 (Biocare, USA) was used for immunoreactive reaction with Ki-67 expressed tumour cells. The prognosis of OSCC sample was reviewed according to the Ki-67 expression level, and stated as positive when the nucleus of tumour cells were brownish. The immunochemical staining results were then compared to lymphoid tissue as a negative control. The positive degree of Ki-67 immunoreactivity was reviewed on the quantity and intensity and calculated of each 1000 tumour cells using a 40 X magnification light microscopy CX21 (Olympus America Inc.Melville, NY 11747). Microphotography of some tissue samples was taken by Olympus U-CMAD3 (T2 Tokyo, Japan). The quantity of Ki-67 expression were scored as follows: score-1 for positive cells < 20%; score-2 for positive cells 21% - 50%; score-3 for positive cells 51% -80%; and score-4 for positive cells > 80%.[15] On the other hand, the intensity of Ki-67 expression was considered a score-1 for weak intensity, the score-2 for moderate-intensity, and

a score-3 for strong intensity.[15,16] All data obtained were analysed using regression-correlation (SPSS version 21).

RESULTS

Of the 20 OSCC cases consisted of 8 males and 12 females, with average median age was 49 years old (ranged 20 - 78 years old). The location of tumours were as much as 8 cases in the tongue, 5 cases in gingival, 2 cases in buccal mucosa, 4 cases in the mouth floor, and 1 case in the palate. OSCC grade I found in 7 cases, grade II in 5 cases, and grade III in 8 cases.

Ki-67 expression varied from less than 20% till more than 80%, there were 7 cases (< 20%), 5 cases (21% -50%), 2 cases (51% -80%) and 6 cases (> 80%). While Ki-67 intensity was found to be weak in 6 cases, moderate-intensity in 7 cases, and strong intensity in 7 cases (Table 1.) An example of Ki-67 immunoexpression was shown in Figure 1. The statistical analysis showed that there is a significant correlation between Ki-67 expression and histopathology grade (r = 0952). The stronger intensity value of Ki-67 expression, then the higher histopathology grade (p < 0.01).

Table 1. Ki-67 expression and intensity on each histopathological grade of OSCC

Histopathology grade	n	Expression (%)				Intensity			P
		<20	20-50	51-80	>80	Weak	Moderate	Strong	
I	7	5	2	-	-	6	1	-	
II	5	2	3	-	-	-	5	-	<0.01
III	8	-	-	2	6	-	1	7	

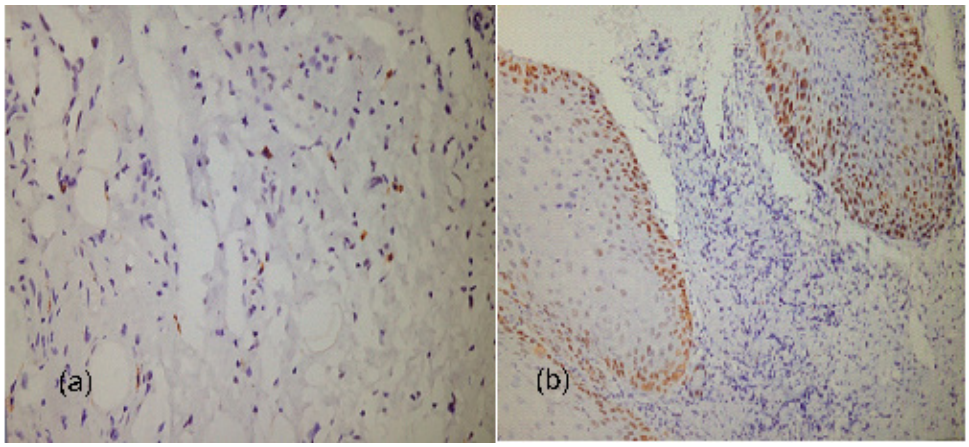

Figure 1. Immunoexpression of Ki-67 in oral squamous cell carcinoma with (a) weak intensity and (b) strong intensity

DISCUSSION

OSCC, as well as other tumours, composed of various cells with different biological properties, so the clinical parameters and histopathological grading alone cannot determine the biological behaviour or the prognosis of the tumour.[6] Some types of tumours may develop highly as a result of the proliferation rate of cells, while in other tumour types, the development of a tumour due to mutations in genes that regulate apoptosis causing progression of the tumour. The occurrence of malignancy, in fact, can be affected by genes. Normally, genes stimulate cell growth and regulate cell apoptosis. Mutation of genes that regulate apoptosis, it could be resulting in excessive cell proliferation and lack of apoptosis. The tumour is final result of excessive cell proliferation compared to the death cells.[3,5]

In all 20 cases of OSCC studied are grouped by grade I, II and III. Generally show that the expression of Ki-67 increased and its associated very strongly to the increase of histopathological grading. The degree of proliferation of a tumour is closely related to the biological behaviour of the tumour. The faster and higher tumour proliferation, the more aggressive tumour and the worse prognosis.[15,17,18]

The used of antibody Ki-67 in this study proven that cell proliferation can be detected

because the antibody will only be expressed at proliferative cells. As it has been explained that Ki-67 antibody is expressed in the cell nucleus proliferating phase which are the G1 phase, S phase, G2 phase and M phase, and not expressed in a resting phase (G0 phase) of the cell cycle.[5,19] Various proliferation molecular markers have been identified, and their relation to the development of tumours of the oral cavity has a lot discussed.[6,10] Also, it is known that the use of one type of oncoprotein is not a valuable biological indicator in predicting prognosis for the patient. While using various markers to determine the behaviour of the tumour is more accurate.[20,21]

Our results were consistent with the research earlier which connects the Ki-67 Labeling index (LI) with histopathological grading according to Broder's. This result indicated that the histopathological grading according to Broder's is very subjective.[18,22] Other studies assessed the relationship Ki-67 with prognostic value in 28 cases of squamous cell carcinoma of the oral cavity, found no significant correlation between Ki-67 with histopathological grading.[17,23] While one study stated that immunoexpression of Ki-67 is useful in predict changes in a dysplasia into malignant.[24] One researcher also explains that the Ki-67 is useful for predicting recurrence in the treatment of squamous cell carcinoma of the tongue in early stages.[25] However, one other study pointed out that the expression of Ki-67 was higher in patients with squamous cell carcinoma with age more than 50 years. In older age patients, the rate of tumour proliferation becomes higher and more aggressive.[26]

CONCLUSION

In conclusion, there is a significant correlation between immunoexpression Ki-67 and the histopathological grade I and III in OSCC; thus, Ki-67 can be used to determine the behaviour and prognosis of OSCC.

ACKNOWLEDGEMENT

There are no competing interests in this study, and this study was funded by DIPA 2015 of the Faculty of Dentistry Universitas Padjadjaran, Indonesia.

REFERENCES

1. Regezi JA, Sciubba JJ, Jordan RCK. Oral Pathology: Clinical Pathologic Correlations. 5th ed. St. Louis, MO: Elsevier; 2008.
2. Oliveira LR, Ribeiro-Silva A. Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2011 Mar;40(3):298-307. DOI: 10.1016/j.ijom.2010.12.003
3. Arul ASKJ, Solomon RDJ, Arul ASSJ, Santhi VS. Immunohistochemical evaluation of Bcl-2 and Ki-67 in varying grades of oral squamous cell carcinoma. J Sci Industr Res. 2011 Nov;70(11):923-8.
4. Klimowicz AC, Bose P, Nakoneshny SC, Dean M, Huang L, Chandarana S, et al. Basal Ki67 expression measured by digital image analysis is optimal for prognostication in oral squamous cell carcinoma. Eur J Cancer. 2012 Sep;48(14):2166-74. DOI: 10.1016/j.ejca.2012.04.010
5. Garewal J, Garewal R, Sircar K. Expression of Bcl-2 and MIB-1 Markers in Oral Squamous Cell Carcinoma (OSCC)- A Comparative Study. J Clin Diagn Res. 2014 Jul; 8(7): QC01-QC04. DOI: 10.7860/JCDR/2014/6474.4562
6. Solomon MC, Carnelio S, Gudattu V. Molecular analysis of oral squamous cell carcinoma: a tissue microarray study. Indian J Cancer. 2010 Apr-Jun;47(2):166-72. DOI: 10.4103/0019-509X.63013
7. Yang WCV, Chung HR, Wu JY, Yi C, Wang DJ, Lee SY. Potential biomarkers for the cytologic diagnosis of oral squamous cell carcinoma. J Dent Sci. 2010 Jun;5(2):60-9. DOI: 10.1016/S1991-7902(10)60010-4
8. Freudlsperger C, Freier K, Hoffmann J, Engel M. Ki-67 expression predicts radiosensitivity in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2012 Aug;41(8):965-9. DOI: 10.1016/j.ijom.2012.04.014
9. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013 Jun;139(2):539-52. DOI: 10.1007/s10549-013-2560-8

10. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep. 2015 Mar;11(3):1566-72. DOI: 10.3892/mmr.2014.2914
11. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011; 103(22): 1656-64. DOI: 10.1093/jnci/djr393
12. Dhall D, Mertens R, Bresee C, Parakh R, Wang HL, Li M, et al. Ki-67 proliferative index predicts progression-free survival of patients with well-differentiated ileal neuroendocrine tumors. Hum Pathol. 2012; 43(4): 489-95. DOI: 10.1016/j.humpath.2011.06.011
13. Desmeules P, Hovington H, Nguilé-Makao M, Léger C, Caron A, Lacombe L, et al. Comparison of digital image analysis and visual scoring of KI-67 in prostate cancer prognosis after prostatectomy. Diagn Pathol. 2015; 10: 67. DOI: 10.1186/s13000-015-0294-0
14. Cordes C, Münzel AK, Rudolph P, Hoffmann M, Leuschner I, Gottschlich S. Immunohistochemical staining of Ki-67 using the monoclonal antibody Ki-s11 is a prognostic indicator for laryngeal squamous cell carcinoma. Anticancer Res. 2009; 29(4): 1459-65.
15. Hornick JL. The prognostic role of immunohistochemistry in sarcomas. Companion meeting of the International Society of Bone and Soft Tissue Pathology; 2010 March 21; Washington DC, USA. Georgia: USCAP; 2010.
16. Humayun S, Ram Prasad V. Natl J Maxillofac Surg. 2011; 2(1): 38–46. DOI: 10.4103/0975-5950.85852
17. Xie S, Liu Y, Qiao X, Hua RX, Wang K, Shan XF, et al. What is the Prognostic Significance of Ki-67 Positivity in Oral Squamous Cell Carcinoma? J Cancer. 2016; 7(7): 758-67. DOI: 10.7150/jca.14214
18. Zeggai S, Harir N, Belkacem A, Tou A, Sellam F, Mrabent NM, et al. Clinical-pathological features and histological variants of Hodgkin's lymphoma: a study of 526 patients. Egypt J Haematol. 2016; 41(3): 140-3. DOI: 10.4103/1110-1067.196218
19. Faur AC, Sas I, Motoc AG, Cornianu M, Zamfir CL, Lazăr DC, et al. Ki-67 and p53 immunostaining assessment of proliferative activity in salivary tumors. Rom J Morphol Embryol. 2015; 56(4): 1429-39.
20. Dadfarnia T, Mohammed BS, Eltorky MA. Significance of Ki-67 and p53 immunoexpression in the differential diagnosis of oral necrotizing sialometaplasia and squamous cell carcinoma. Ann Diagn Pathol. 2012; 16(3): 171-6. DOI: 10.1016/j.anndiagpath.2011.08.011
21. Premalatha BR, Uma K. Analysis of KI-67 antigen in human Oral Squamous Cell Carcinoma – an immunohistochemical study. J Int Oral Health. 2010; 2(1): 9-16.
22. Motta RDR, Zettler CG, Cambruzzi E, Jotz GP, Berni RB. Ki-67 and p53 correlation prognostic value in squamous cell carcinomas of the oral cavity and tongue. Braz J Otorhinolaryngol. 2009; 75(4): 544-9. DOI: 10.1016/S1808-8694(15)30494-8
23. Ashraf MJ, Maghbul M, Azarpira N, Khademi B. Expression of Ki67 and P53 in primary squamous cell carcinoma of the larynx. Indian J Pathol Microbiol. 2010; 53(4): 661-5. DOI: 10.4103/0377-4929.72019
24. Wangsa D, Ryott M, Avall-Lundqvist E, Petersson F, Elmberger G, Luo J, et al. Ki-67 expression predicts locoregional recurrence in stage I oral tongue carcinoma. Br J Cancer. 2008; 99(7): 1121–1128.
25. Rodrigues RB, Motta RDR, Machado SMDS, Cambruzzi E, Zettler EW, Zettler CG, et al. Prognostic value of the immunohistochemistry correlation of Ki-67 and p53 in squamous cell carcinomas of the larynx. Braz J Otorhinolaryngol. 2008; 74(6): 855-9. DOI: 10.1016/S1808-8694(15)30145-2