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# Comparative Investigation of Ki67 Marker in Oral Squamous Cell Carcinoma and Oral Lichen Planus by Immunohistochemistry

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**Original Research Article**

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**Abstract:** Oral lichen planus (OLP) is a chronic inflammatory disease with unknown etiology which WHO has classified it as a premalignant lesion that could be transformed to oral squamous cell carcinoma (OSCC). Ki67 marker use for identifying divided cells. Therefore, it shows proliferation activity in the normal and tumoral tissues. The aim of this study was to compare the immunohistochemical expression of Ki67 marker in OLP and OSCC. This study was a Laboratory experimental study. Immunohistochemistry (IHC) expression of Ki67 marker was studied in 30 samples of hyperplasia without dysplasia (control group), oral lichen planus and squamous cell carcinoma. The independent T-test was used to compare the expression levels of Ki-67 marker in OSCC, OLP and hyperplasia. Statistical analysis was performed with SPSS Ver.21 software. The mean stained cells in samples hyperplasia were 19.93±7.5%, OLP were 19.4±7.6% and in OSCC were 75.43±13.1%. There was no significant difference in average expression of Ki67 between hyperplasia samples and OLP ($P>0.05$), but the expression of Ki67 in OSCC was higher than OLP and hyperplasia and this difference was significant ($P<0.01$). The results showed that Ki67 expression in squamous cell carcinoma was higher than oral lichen planus and hyperplasia and more Ki67 expression in samples of OLP demonstrated an increase in the proliferation and progression to malignancy.
**Keywords:** Ki67, oral squamous cell carcinoma, oral lichen planus, Immunohistochemistry

## INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the most common cancers in all over the world [1, 2]. This carcinoma is a malignant and invasive tumor that expression of its new cases will be annually increased and it is resulting in considerable disability and mortality of suffering patients [1]. Despite much progress, the average for its remaining is low and it was not improved in recent decade. Fast and early detection of this tumor seems necessary for performing suitable treatment and preventing in the primary stages of injury expression and improving patient prognosis [2-4].

The most OSCCs will be started following a pre-cancer injury [1]. If these injuries are detected in the primary stages, the probability of malignant expression will be low in them. One of pre-malignant injuries which are subject to malignant change is Oral Lichen Planus (OLP) [5-9]. OLP is an inflammatory mucous dermal disease with unknown reasons [5, 6]. It is mostly seen in people over 40 years and in some cases; the mouth is the only involved zone. Women are suffering with relative ratio 1.4 to 1 more than men and despite skin lesions which have not primarily malignant changes, oral lesions of patients suffering from OLP are subject to converting to OSCC [9, 10].

Carcinogenesis is a multi-stage process that at first starts by increasing proliferation and mutation in genes involved in cell cycle [10]. Cell cycle in different stages is controlled by several genes that disorder and mutation in each one of them causes damage to cell DNA and consequently increasing uncontrolled cell proliferation causes making tumor [10, 11]. Among regulating factors of cell cycle, Ki67 can be mentioned that by matching effective proteins in cell proliferation plays role in different phases of the cell proliferation cycle.

Ki67 is an antigen for detecting cell proliferation and matches nuclear proteins in proliferative phases of cell cycle in all cells. The expression of this antigen indicates the activity of cell proliferation and it has a direct relation to increasing mitotic activity and mitotic count. By investigating the percentage of colored cells by Ki67, the amount of cell proliferation activity can be detected. The tonality of cells with Ki67 confirms more and sometimes

uncontrolled cell divisions and increasing the potential of malignant change. The expression of Ki67 in head and neck tumors is along with decreasing prognosis of suffering patients. Since uncontrolled proliferation of cell is considered as one of the most important carcinogenesis biological mechanisms and changing in the expression of its related proteins is considered as one of the most important indices for detecting potential of malignant change of injuries, in present study by using immunohistochemistry coloring method, the expression of Ki67 was compared and evaluated in OLP and OSCC injuries to predict the probability of future malignant change event in OLP by using the results of this study.

## MATERIALS & METHODS

This study was a retrospective type and descriptive-analytical that was performed by sectional method. After investigating archive of pathology section of dentistry college of Qazvin medical science university, study samples were selected based on case contents and the pathology report of patients. Then all samples were reviewed by two pathologists. It is necessary to note that for confirming detection of OLP injuries, defined criteria was used by Eisenbrg. The samples with having dysplasia changes, suspected diagnosis with inadequate proving, hemorrhage and necrosis were excluded. Also the registration of the risk of interfering factors such as cigarette, alcohol and drug consumption in the case or concurrent detection of other injuries (along with main samples) was among the criteria for exclusion of study.

90 samples consisting epithelial hyperplasia injuries (control group) of Lichen Planus and Oral Squamous Cell Carcinoma were selected. Selected hyperplasia samples were without dysplasia and immunohistochemistry coloring method of samples was prepared with Ki67 antibody (Denmark DAKO, ready-to-use, code N1385) and it was performed by peroxide method from anti peroxide as follows:

Four micron cut was prepared from each sample and put on prepared lams. Lams were put in 58C temperature in oven for 24 hours and then they were passed from two Xylene dishes for five minutes for the purpose of paraffin destruction and also alcohol with different degrees (up to 70%) for watering. After washing with distilled water in next stage for antigen recycle of lams, it was put in buffer citrate solution with PH=9. At first this collection was put in the microwave for five minutes with 80w and then 15 minutes with 450w. After washing lams (for 15 minutes) and drying for preventing peroxide, all samples were incubated in peroxide hydrogen from 3% for 20 minutes. After this stage and in the distance of next stages which were adding primary antibody, secondary antibody, DAB and haematoxylin for coloring was used for washing Phosphate buffered saline: PBS, respectively. On the final stage, samples were put in alcohol with different degrees for watering and then Xylene for clarification and finally they were mounted. In all stages, Mantle cell lymphoma sample was used for positive control for ensuring the accuracy of coloring.

Core was used for ensuring the accuracy of coloring. The core of colored cells with antibody (browned cores) was used as coloring. The core of colored cells with antibody (browned cores) was considered as positive tonality. For counting colored cells with Ki67, optical microscope (Olympus BX41, Tokyo, Japan) was used. At first lams was observed in low magnification (x40) and the zones were determined with the maximum tonality. Then epithelial was counted and the percentage of colored cells of Label INDEX: L1 was calculated for each sample (quantitative survey). Also the presence of Ki67 marker was investigated by semi-quantitative method. Hence the number of colored cells was ranked and studied to less than 1% colored types equals 0, 10%-1 equals +1, 35%-10 equals +2, 70%-35 equals +3 and the samples more than 70% coloring as +4.

Gathered information was analyzed by using SPSS Ver.16 software and statistical tests were one way ANOVA and Tukey (for comparing quantitative variables among groups), krurskal-wallis and Mann-Whitney (for comparing semi-quantitative variables among groups) and nonparametric Fisher test (for comparing qualitative variables among groups). Significant level was <0.05 in all tests.

## RESULTS

In investigating immunohistochemistry coloring, nuclear tonality was observed with Ki67 indicator (positive tonality) in all groups (100%). Cell count was performed based on what was explained in the materials and methods section. The minimum and maximum colored cells in hyperplasia samples were 7.8 and 37.1 in OLP samples and OSCC samples 50.7 and 99.4, respectively (Table 1, Figure 1).

**Table -1: Descriptive parameters of number of stained cells in the groups**

| Group | Number of samples | Minimum | Maximum | Mean | Standard deviation |
|---|---|---|---|---|---|
| Epithelial hyperplasia | 30 | 7.8 | 37.1 | 19.93 | 7.5 |
| Oral Lichen Planus | 30 | 7.3 | 36.6 | 19.4 | 7.6 |
| Squamous cell carcinoma | 30 | 50.8 | 99.4 | 75.43 | 13.1 |

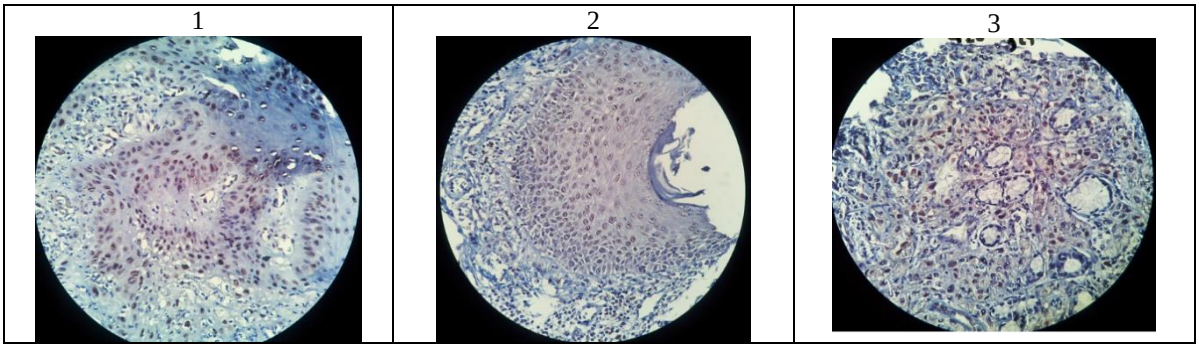


**Fig-1: Stained samples by Ki67: OLP (1), Hyperplasia (2), OSCC (3) in ×100**

The average percentage for the presence of Ki67 in the hyperplasia epithelial group was 19.93± 7.5, in OLP group 19.4± 7.6 and in OSSCC group 75.43± 13.1. The ANOVA showed that there was a significant difference in terms of Ki67 in studying groups (P<0.001). Also by using TUKEY test, the average of staining percentage Ki67 in the pairwise investigation of groups had a significant statistical difference (P<0.001).

The results related to the study of Ki67 indicator with semi-quantitative method (ranking) in groups were shown in table 2. There was significant statistical difference in terms of ranking of Ki67 marker among three studying groups (Kurskal Wallis test P<0.001). This significant statistical difference was seen in a pairwise survey among groups (Mann-Whitney, p <0.05).

**Table-2: Frequency of the Ki67 marker ratings in the groups on the base of the positive epithelial cells**

| Group | Number of samples | 0 <1% | +1 1-10% | +2 10-35% | +3 35-70% | +4 >70% |
|---|---|---|---|---|---|---|
| Epithelial hyperplasia | 30 | 0 (0%) | 7(27.5%) | 22(71.5%) | 1(1%) | 0 (0%) |
| Oral Lichen Planus | 30 | 0 (0%) | 6(20%) | 23(79%) | 1(1%) | 0 (0%) |
| Squamous cell carcinoma | 30 | 0 (0%) | 0 (0%) | 0 (0%) | 10(33.3%) | 20(66.6%) |
| Total | 90 | 0 (0%) | 13(14.1%) | 45(50%) | 22(18.4%) | 20(17.5%) |

It is necessary to note in two tables, less than 35% staining was observed in all cases of epithelial hyperplasia and OLP (100% samples), while all samples of OSCC group (100% cases), was shown the staining more than 35%.

## DISCUSSION

The final purpose of this study was the comparison of expression amount of Ki67 marker in the samples of oral squamous cell carcinoma, oral lichen planus and hyperplasia samples with immunohistochemistry. In the present study, the average of colored cells in the samples of hyperplasia was 19.93±7.5 percent, in OLP samples 19.4±7.6 percent and in the samples of OSCC 75.43±13.1 percent. In the comparison of percentage average of Ki67 hyperplasia with OLP, there was no significant difference (P> 0.05). The result of Renata study *et al*. [35] was consistent with the result of the present study. They showed in the evaluation of Ki67 marker in OLP samples and oral lichenoid injuries (as control) that by Ki67 expression, there was a significant difference between these two injuries. But the result of the study of Taniguchi *et al*. [25] in using Ki67 was not consistent with the results of the present study for investigating the proliferation of epithelial cells in OLP injuries. They reported that the amount of Ki67 expression in OLP samples was significantly higher than the normal mucous membrane. Also the result of the study of Raju *et al*. [21] in investigating p53, Ki67 and cyclin D1 in oral premalignant and malignant injuries showed that increasing cyclin D1, p53 and Ki67 in malignant injuries was respectively 45.5%, 100% and 80% in comparison with 82.8%, 65.5% and 85.1% of premalignant injuries. The result of the study of Flatharta *et al*. [18] was not consistent with the result of the present study. In investigating ji67 in OLP, they reported that there was statistically significant increasing between Ki67 notes in OLP samples in comparison with normal mucous membrane. Hadzi *et al.* [37] in investigating Ki67 expression in OLP in comparison with Ki67 expression in healthy people reported that Ki67 in lymphocytes and keratinocyte of OLP patients was more in comparison with healthy people. Among other studies, non-consistent with the result of the present study, the results arising Agha-Hosseini *et al*. [38] and Pigatti *et al*. [2] were that the amount of Ki67 expression in OLP injury was more than control cells. This change in results probably can be related to the number of sampling and the stage of injury growth, which in the present study was different with other studies. Ki67 may not be as a marker for

determining pre-awareness in developing the treatment of OLP patients, but it can help in selecting patients who need more following for preventing malignancy. In general increasing cell proliferation in OLP was introduced independently from mutagenic changes and secondary phenomenon to inflammatory infiltration and keratinocyte damages. Hence Rajentharan *e al*. [34] proposed that although reported malignant changes in OLP injuries are not high, but clinicians should consider the possibility of malignant change in these injuries and perform the least six year follow up after detecting Lichen Planus.

In the present study in comparison with the average of hyperplasia Ki67 and OLP with OSC, there was a significant difference (P<0.01). The average of Ki67 expression in OSCC samples was more than hyperplasia and OLP samples. The result of the study of Jalayer Naderi *et al.* [36] was consistent with the result of the present study. In investigating the expression of Ki67, P53 protein and C-erb-B2 antigen indices and their relationship in the cancer of Oral Squamous Cell, they showed that the expression of three indices in Oral Squamous Cell was high. The result of the study of Hadzi *et al*. [37] was consistent with the results of the present study. In evaluating the expression of Ki67 in healthy people in comparison with patients suffering from OLP and OSCC with IHC method, they reported that the amount of expression of this gene in patients suffering from OSCC was more than healthy people and OLP. The result of the study of Ali Khasi *et al.* [3] was consistent with the result of the present study. In a comparative investigation of the expression of D1 cyclin in Lichen Planus, Oral Squamous Cell Carcinoma and epithelial hyperplasia samples (control group) by immunohistochemistry method, they reported that patients suffering from OLP are in danger of more suffering to OSCC, the results of this research with cyclin D1 indicate cell proliferation in OLP injuries was significantly less than OSCC samples. This issue is a warning for clinicians that patients suffering from OLP especially that category which has increased cell proliferation will be always followed by regular cycle and continuous examinations until the smallest change on their injuries will be determined in the same primary stages and suitable treatment is done. The highness of Ki67 expression in OSCC in fact indicates higher proliferation and the mutation of suppressor tumor.

In the process of cell proliferation, there is need for cell division that is based on the control of noted molecules during cell cycle including Ki67. The lack of balance or increasing cell proliferation in different injuries such as cancer and cyst is reported and the amount of injuries, growth is one of detection ways for their offensive state [42]. Based on previous studies and present study, we can say that Ki67 is a type of marker of cell proliferation that can be used as Oral Squamous Cell Carcinoma prognosis.

## CONCLUSION

The results showed that Ki67 expression in squamous cell carcinoma was higher than oral lichen planus and hyperplasia and more Ki67 expression in samples of OLP demonstrated an increase in the proliferation and progression to malignancy.

## REFERENCES

1. Farhadi S, Abass Mashhadi F, Hadshi R. Review of Relation between P53 & OSCC lesions prognosis. Journal search in dentistry sience. 2013; 2(36):15-19.
2. Pigatti FM, Tareira LA, Soares CT. IHC expression of Bclz and Ki67 in oral licken planus and leukoplakia with different degrees of dysplasia. Journal of ludian society of periodontology 2012; 16 (2) 253.
3. Ali khasi M, Jamshidi S, Zargaran M. Comparative Investigation of Cyclin D1 Expression in Oral Lichen Planus and Squamous Cell Carcinoma by Immunohistochemistery Technique. Journal Management System.2014; 17-28
4. Jalayer Naderi N, Eslami M, Sepehr Ara A. unicystic ameloblastoma: Clinico-pathological study of 52 cases. Daneshvar, 2007, 15(17): 17-24.
5. Sirajdarss, Radhika M, Paremalak. Expression of Ki67 in normal oral epithelium, leakoplic oral epithelium and oral squamous cell carciuma. Journal oral Maxillofacpathol. 2014; 18 (2): 169-76.
6. 6. Luis Monteriro, MarcioDiniz, Freitas. Tomas Garcia-Caballero. EGFR and Ki67 expression I oral squamous cell carsinouma using tissue microarray technology. Journal of oral pathology and Medicine. 2010; 39 (7):18-21.
7. Cawson RA, Odell Ew. Oral pathology and oral medicine. Sounder co, 2008:277-286.
8. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral & Maxillofacial Pathology. 5ed: Saunders co; 2008. 594-597,611-618.
9. Robbins, Vinay kamar, standy Leonard, Basic pathology, 8 th, 2007: 573-574.
10. Regezi JA, Sciubba JJ, Jordan RCK. Oral pathology- clinical pathologic correlation. 4ed; 2008.250-260.
11. Greenburg M, Glick M, Ship J, Burket, lester William, Burket's oral medicine, 11 th, 2008: 170-180.
12. James OD, Gee MC, peten G. Issascon, pathology of system 2a. 2th, 1992: 1059-1066.
13. White E starts C. oral radiology perinciples and interpretation. Sounder co, 5 th 2004: 488: 490-491.
14. Fillies T, Wermeister R, Diest J, Joos h, Burkhrd B. HIFI-alpha overexpression indicate a good prognosis in early stage squamous cell

carcinomas of the oral floor. BMC cencer 2009; 5:84-87
15. Rubin E, Aaronson S, Neoplasia. In: Rubin E, Gorstein F, Rubin R, Schwarting R, Strayer D, editors. Rubin's pathology clinicopathologic fondations of medicine. 4th ed. 2004. P 83.
16. Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus--a review. J Oral Pathol Med 2010; 39:729-34.
17. Ma LW, Zhou ZT, He QB, Jiang WW. Phospholipase C-γ1 expression correlated with cancer progression of potentially malignant oral lesions. Journal of Oral Pathology & Medicine. 2013 Jan 1;42(1):47-52.
18. Flatharta C, Flint W, Toner M, Mabruk M. hTR RNA Component as a Marker of Cellular Proliferation in Oral Lichen Planus. Asian Pacific JCancer Prey, 9, 287-290
19. Mattsson U, Jontell M, Holmstrup P. Oral lichen planus and malignant transformation: is a recall of patients justified? Critical Reviews in Oral Biology & Medicine. 2002 Sep;13(5):390-6.
20. Montebugnoli L, Venturi M, Gissi S, Leonardi E, Farnedi A, Maria P .Immunohistochemical expression of p16INK4A protein in oral lichen pianus. Oral Oncology 2007; 42: 4 75-480
21. Raju B, Mehrotra R, Øijordsbakken G, Al-Sharabi AK, Vasstrand EN, Ibrahim SO. Expression of p53, cyclin D1 and Ki-67 in pre-malignant and malignant oral lesions: association with clinicopathological parameters. Anticancer research. 2005 Nov 1;25(6C):4699-706.
22. Lavanya N, Jayanthi P, Rao UK, Ranganathan K. Oral lichen planus: An update on pathogenesis and treatment. Journal of oral and maxillofacial pathology: JOMFP. 2011 May;15(2):127.
23. Gándara-Rey JM, Freitas MD, Vila PG, Carrion AB, Penaranda JM, Garcia AG. Malignant transformation of oral lichen planus in lingual location: report of a case. Oral Oncology EXTRA. 2004 Jan 31;40(1):1-4.
24. Cortés-Ramírez DA, Rodríguez-Tojo MJ, Gainza-Cirauqui ML, Martínez-Conde R, Aguirre-Urizar JM. Overexpression of cyclooxygenase-2 as a biomarker in different subtypes of the oral lichenoid disease. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010 Dec 31;110(6):738-43.
25. Taniguchi Y, Nagao T, Maeda H, Kameyama Y. Epithelial cell proliferation in oral lichen planus. Cell Prolif 2002; 35:103-109.
26. Turatti E, Adriana D. Assessment of c-Jan, c-fos and cyclin D1 in premalignant and malignant oral lesions. Journal of oral science, 2005;47: 71-76.
27. Kademani D, Lewis JT, Lamb DH, Rallis DJ, Harrington JR. Angiogenesis and CD34 expression as a predictor of recurrence in oral squamous cell carcinoma. Journal of Oral and Maxillofacial Surgery. 2009 Sep 30;67(9):1800-5.
28. Bankfalvi A, Piffko J. Prognostic and predictive factors in oral cancer: the role of the invasive tumour front. Journal of oral pathology & medicine. 2000 Aug 1;29(7):291-8.
29. Kurokawa H, Zhang M, Matsomoto SH. The relationship of the histological grade at the deep invasive front and the expression at Ki-67 antigen and P53 protein in oral squamous cell carcinoma. J. oral. Pathol. Med. 2005, 34: 602-607.
30. Tumuluri V, Thomas GA, Fraser IS. Analysis of the Ki-67 antigen at the invasive tumor front of the human oral squamous cell carcinoma. J. oral. Pathol. Med. 2002, 31: 598-604.
31. Piffko J, Bankfalvi A, Ofner D. in situ assessment of cell proliferation at the invasive front of oral squamous cell carcinoma. Virchows Arch. 1996, 429: 229-234.
32. Allison RT, Best T. P53, PCNA and Ki-67 expression in oral squamous cell carcinoma, the vagaries of fixation and microwave enhancement of immunocytochemistry. J. oral. Pathol. Med. 1998, 27: 434-440.
33. Mostafa WZ, Mahfooz S, Bosseila M. An immunocytochemical study of laminin in cutaneous & mucosal squamous cell carcinoma. J. Egypt Wom Dermatol. Soc. 2007, 4(1): 432-439.
34. Rajentheran R, McLean NR, Kelly CG, Reed MF, Nolan A. Malignant transformation of oral lichen planus. European Journal of Surgical Oncology (EJSO). 1999 Oct 31;25(5):520-3.
35. Renata Rodrigues A, Claudia Ronca F. Euluation of proliferative potential in oral lichen planus and oral lichenoid lesions using immunohistochemical expression of p53 and Ki67 .oral oncology2006;8: 475-480.
36. Jalayer Naderi N, Tirgari F, Gholamalipoor N, Journal deneshvar. 2008; (1-3).
37. Hadzi-Mihailovic M. Ki67 expression in oral licken plaus. Journal of B. V. on; official journal of the Balkan union of oncology 2008; 17(1): 133-7.
38. Agha-Hosseini F, M Khalili, B Rohani. IHC Analysis of P53 and Ki67 proteins in oral Licken Planus and Normal oral Mucosa. Irannian Journal of Public Health, Monthly. 2009; 38 (2) 37-43.
39. Bombeccari GP, Guzzi G, Tettamanti M, Giannì AB, Baj A, Pallotti F, Spadari F. Oral lichen planus and malignant transformation: a longitudinal cohort study. Oral Surgery, Oral

Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2011 Sep 30;112(3):328-34.

40. Salehinejad J, Sharifi N, Amirchaghmaghi M, Ghazi N, Shakeri MT, Ghazi A. Immunohistochemical expression of p16 protein in oral squamous cell carcinoma and lichen planus. Annals of diagnostic pathology. 2014 Aug 31;18(4):210-3.
41. Schlephaka H. Prognostic relevance of molecular markers of oral cancer-a review. Int J Oral Maxillofac Surg., 2003; 32(3): 233-45.
42. Schlephaka H. Prognostic relevance of molecular markers of oral cancer-a review. Int J Oral Maxillofac Surg., 2003; 32(3): 233-45.