DNA Fragmentation and mRNA Expression of Bcl-2, Bcl-xL, p53, p21 and HSP70 Genes in Nondysplastic and Dysplastic Oral Lichen Planus

Abstract

Background: Oral lichen planus (OLP) is a chronic inflammatory disease. Apoptosis of the basal keratinocytes is a causative factor for OLP pathogenesis but the detailed mechanism of apoptosis among nondysplastic and dysplastic OLP lesions is yet unraveled. **Aims:** This study aims to evaluate the involvement of cellular DNA fragmentation and alteration in the expression of Bcl-2 and B-cell lymphoma extra-large (Bcl-xL), p53, p21 and heat shock protein 70 (HSP70) in nondysplastic and dysplastic OLP lesions. **Materials and Methods:** Untreated, fifteen OLP patients each with nondysplastic and dysplastic lesions were enrolled for this study. Their DNA fragmentation was analyzed by the agarose gel electrophoresis method. The mRNA expression of Bcl-2, Bcl-xL, p53, p21 and HSP70 were measured using semi-quantitative reverse transcription-polymerase chain reaction. **Results:** Elevated DNA fragmentations were found in dysplastic lesions compared to nondysplastic type. Significantly higher expression of Bcl-2, Bcl-xL, p53 and p21 were found in both types of OLP lesion compared to the control. Expression of Bcl-2 and Bcl-xL were significantly elevated in nondysplastic lesions, whereas significantly overexpression of p53 and p21 were found in dysplastic lesions. Anti-stress protein HSP70 was overtly expressed in dysplastic lesions compared to other groups. **Conclusion:** Reduced expression of Bcl-2 and Bcl-xL, with elevated DNA fragmentation, may be associated with increased apoptosis in dysplastic lesions which aid in the resolution of the chronic inflammatory process. Higher expression of p53 and p21 in dysplastic lesions reflect its malignant potentiality. Overexpression of HSP70 in dysplastic lesions is a useful marker for higher cellular stress.

Keywords: *Apoptosis, DNA fragmentation, dysplasia, malignant potentiality, oral lichen planus*

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Introduction

Lichen planus is a chronic inflammatory disease condition that affects the skin and the oral mucus membrane. The etiology and detailed pathogenesis of oral lichen planus (OLP) has not yet understood.[1] Clinically, OLP appears as white striations (Wickham's striae), white papules, white plaque, erythema, erosion or blisters.[2] It has several subtypes including reticular, erosive, atrophic, papular, plaque-like and bullous and some variants may co-exist.[3] Apoptosis is frequently found in OLP but the mechanism yet to be clarified.[4] It was found that apoptosis prevents aneuploidy and other genetic alterations which are associated with the development and progression of precancerous lesions.[5] Among the other important markers of apoptosis, DNA fragmentation is considered a

hallmark.[6] OLP has a higher risk of malignant transformation compared to the cutaneous lesion, the reported range being 0%–12.5%.[7] The presence of oral epithelial dysplasia is an important predictor for malignant transformation in oral premalignant disorders like OLP.[8]

Expression of pro-and anti-apoptotic proteins are important to understand the nature of oral lesion and the process in disease progression.[9] Among others, proapoptotic Bcl-2 associated X protein, Bcl-2 associated agonist of cell death, Bcl-2 related ovarian killer, annexin, anti-apoptotic Bcl-2 and B-cell lymphoma extra-large (Bcl-xL) have an important regulatory role in oral lesions formation.[10] Cellular stress produced by DNA-damaging agents can activate apoptosis by the interplay of pro- and anti-apoptotic Bcl family proteins.[11] There is a strong correlation between the expression of p53 and Bcl-2 proteins and neoplastic

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transformation.[12] Heat shock protein 70 (HSP70) is a major stress-inducible protein involved in the protection of cells from many apoptotic stimuli.[13] HSP70 might be a promising molecule for controlling apoptosis.[14]

DNA fragmentation and role of anti-apoptotic Bcl-2, Bcl-xL, cell cycle regulator p21, anti-stress HSP70 gene expression in pathophysiological alteration of nondysplastic and dysplastic OLP lesions formation have not yet looked into. Hence, the present study aims to analyze the DNA fragmentation and alteration in mRNA expression of Bcl-2, Bcl-xL, p53, p21 and HSP70 in nondysplastic and dysplastic OLP lesions. This study may be useful to identify the causative factors of nondysplastic and dysplastic OLP lesion formation.

Materials and Methods

Patient selection

Freshly diagnosed and untreated 15 OLP patients (without any dysplasia) and another 15 OLP patients (with mild-to-moderate dysplasia) aged 35–55 years were enrolled for this study. A total of 15 age and sex matched healthy individuals were recruited as control subjects. The patients were clinically and histologically diagnosed according to modified WHO criteria.[15] Punch biopsy technique was used to collect the OLP lesion tissue samples from the Department of Oral Medicine and Radiology, PMS College of Dental Science and Research, Kerala, India. Normal healthy samples were the discarded tissues from surgical treatments of the impacted tooth, where the normal mucosal tissue margins were trimmed (excised) and discarded to facilitate primary closure, which were collected from the aforesaid department. The data and tissue sample collection were approved by the Institutional Ethical Committee and written consent for publication was obtained.

Exclusion criteria

OLP patients who had undergone any treatment for the same, those previously with habits of chewing or smoking tobacco and those with a history of alcohol consumption were eliminated from this study. OLP patients or normal individuals having asthma, hypertension, diabetes, cardiac disorder, bleeding or clotting disorders, psychiatric illness, hepatitis, AIDS or malignancy were not included in this study.

DNA fragmentation were analyzed by agarose gel electrophoresis method

25 mg tissue was placed in a sterile microcentrifuge tube, 180 μl PureLink™ genomic digestion buffer, and 20 μl proteinase K. The mixture was incubated at 55°C with occasional vortexing until lysis was complete (1–4 h). The lysate was centrifuged at maximum speed for 3 min at room temperature and the supernatant was transferred to a new sterile tube. 20 μl RNase A was added to the lysate, mixed well and incubated at room temperature for 2 min. 200 μl PureLink™ genomic lysis/binding buffer was added and mixed well by vortexing to yield a homogeneous solution. 200 μl 96%–100% ethanol was added to the lysate and mixed well to obtain a homogeneous solution.

Determination of mRNA expression by two-step semi-quantitative reverse transcription-polymerase chain reaction

The mRNA expression of Bcl-2, Bcl-xL, HSP70, p53, p21 and GAPDH in OLP lesion groups and control tissue were studied by using reverse transcription-polymerase chain reaction (RT-PCR) method. Total RNA was isolated from oral tissue using the TRIzol reagent (Invitrogen, USA) according to the manufacture's instruction. RT-PCR was performed using primer designed specifically for the genes to be amplified [Table 1]. cDNA was synthesized using ThermoScript™ kit (Invitrogen, USA). The synthesized cDNA was amplified using Platinum Taq DNA polymerase kit (Invitrogen, USA). The stained gel was observed using the E-gel imager system (Invitrogen, Life Technology, USA). Agarose gel pictures were analyzed by Image J software.

Statistical analysis

The results were expressed as mean ± standard deviation and the statistical analyses were performed using one-way ANOVA and Tukey's *post hoc* test by SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ has been considered as statistically significant.

Results

A cross-sectional study was performed on 15 OLP patients with oral dysplasia and another 15 OLP patients without any dysplasia. The clinical appearance, site of OLP lesions and type of dysplasia in OLP patients have been tabulated [Table 2]. The qualitative analysis of DNA fragmentation was used to detect apoptosis in both types of OLP lesions and healthy control. The mRNA expression of

Table 1: Gene specific forward and reverse primer sequences of apoptosis associated genes

Gene	Primer sequence
Bcl-2	Forward 5'-CCTGTGGATGACTGAGTACC-3'
	Reverse 5'-GAGACAGCCAGGAGAAATCA-3'
Bcl-xL	Forward 5'-GAGTTCATTCACTACCTGTTC-3'
	Reverse 5'-CTGGTGGTTGACTTTCTCTC-3'
p53	Forward 5'-CCACCATGAGCGCTGCTCA-3'
	Reverse 5'-GCAGGGGAGGGAGAGATG-3'
p21	Forward 5'-GCAGATCCACAGCGATATCC-3'
	Reverse 5'-CAACTGCTCACTGTCCACGG-3'
HSP70	Forward 5'-CCATGGTGCTGACCAAGATGAAG-3'
	Reverse 5'-CACCAGCGTCAATGGAGAGAACC-3'
GAPDH	Forward 5'-GAAGGTGAAGGTCGGAGTC-3'
	Reverse 5'-GAAGATGGTGATGGGATTTC-3'

Table 2: Basic characteristics of patients with non-dysplastic and dysplastic changes

	Nondysplastic OLP lesions				Dysplastic OLP lesions				Type of dysplasia
Case number	Gender	Age	Site of lesion	Clinical appearance	Gender	Age	Site of lesion	Clinical appearance	
1	Female	36	Buccal mucosa	Reticular	Female	48	Buccal mucosa	Erosive	Moderate
2	Male	46	Buccal mucosa	Atrophic	Female	53	Buccal mucosa	Erosive	Mild
3	Female	39	Lateral border of tongue	Reticular	Female	55	Buccal mucosa	Plaque	Mild
4	Female	41	Buccal mucosa	Reticular	Female	44	Lateral border of tongue	Erosive	Moderate
5	Female	52	Buccal mucosa	Atrophic	Female	38	Floor of mouth	Erosive	Mild
6	Male	37	Floor of mouth	Atrophic	Male	42	Lateral border of tongue	Plaque	Mild
7	Male	36	Buccal mucosa	Erosive	Male	54	Buccal mucosa	Erosive	Moderate
8	Male	35	Lateral border of tongue	Reticular	Male	36	Buccal mucosa	Plaque	Mild
9	Female	42	Buccal mucosa	Reticular	Female	45	Buccal mucosa	Erosive	Moderate
10	Female	50	Buccal mucosa	Atrophic	Female	49	Lateral border of tongue	Plaque	Mild
11	Female	47	Floor of mouth	Reticular	Male	37	Lateral border of tongue	Erosive	Moderate
12	Male	44	Buccal mucosa	Reticular	Male	51	Buccal mucosa	Plaque	Mild
13	Female	43	Buccal mucosa	Popular	Female	38	Lateral border of tongue	Plaque	Mild
14	Female	51	Lateral border of tongue	Reticular	Male	53	Buccal mucosa	Erosive	Moderate
15	Male	41	Floor of mouth	Erosive	Male	47	Buccal mucosa	Erosive	Mild

OLP: Oral lichen planus

Bcl-2, Bcl-xL, p53, p21 and HSP70 were compared among nondysplastic, dysplastic OLP lesions and control samples.

The presence of saw-toothed rete ridge, Max-Joseph space, and inflammatory infiltration was identified in normal, mild and moderate dysplastic OLP lesions. Among other identifying characters, hyperchromatic nuclei were commonly observed in mild and moderate dysplastic lesions. In addition, increased nucleocytoplasmic ratio and enlarged nucleoli were found in mild dysplastic lesions, whereas, mitotic figure and basal cell hyperplasia were seen in moderate dysplastic lesions [Figure 1].

DNA fragmentation is a classic feature of apoptosis, cellular DNA is broken down into smaller fragments by the enzymes which are activated under apoptotic stimuli. This DNA fragmentation produces a characteristic pattern that can be visualized by gel electrophoresis, termed as DNA laddering. Higher fragmented DNA patterns were observed in mild and moderate dysplastic OLP lesions as compared with nondysplastic and healthy controls [Figure 2].

The mRNA expression of Bcl-2 ($P < 0.001$), Bcl-xL ($P < 0.0001$), p53 ($P < 0.0001$) and p21 ($P < 0.0001$) significantly differed from the control, nondysplastic and dysplastic OLP lesion group patients. Significantly higher expression of Bcl-2 and Bcl-xL were found in OLP groups compared to the control, whereas, expression of both anti-apoptotic genes were significantly ($P < 0.05$) elevated in nondysplastic lesions compared to the dysplastic type. Expression of transcription factor p53 and cell cycle regulator p21 were significantly elevated in OLP groups compared to control. Significantly higher expression of p53 ($P < 0.0001$) and p21 ($P < 0.006$) were found in dysplastic lesions compared to nondysplastic OLP. Importantly, significant overexpression of HSP70 ($P < 0.0001$) was seen in dysplastic OLP lesions when compared with nondysplastic and control tissue [Figure 3].

Discussion

OLP is a T-cell-mediated autoimmune disease of unknown etiology. Apoptosis of basal keratinocytes of the oral epithelium initiates the pathogenesis OLP.[16] The stimulators for apoptosis in OLP are still unknown, in which auto-cytotoxic CD8+ T cells attack the basal epithelial cells and activate vital molecular mechanisms namely cell cycle arrest for DNA repair and induce cell senescence or apoptosis to eliminate cells with severely damaged DNA.[17] DNA fragmentation is the main characteristic feature of apoptosis and thus is used as an important marker of apoptosis.[18] In the present study, DNA fragmentations in dysplastic lesions were higher compared to nondysplastic and control type which is an indicator for the high rate of apoptosis in dysplastic lesions.

The growth of oral keratinocytes is regulated by a delicate balance between anti-apoptotic Bcl-2 that controls cell survival and p53 that controls cell death.[19] Alteration in the expression of these proteins is a strong indicator of the malignant transformation potential of a certain lesion.[20] The overexpression of the Bcl-2 and Bcl-xL were found to protect the cells from undergoing apoptosis in response to a number of apoptotic stimuli.[21] The detailed mechanism by which Bcl-2 inhibits apoptosis is still uncertain, it was suggested that Bcl-2 and Bcl-xL may inhibit mitochondrial cytochrome c translocation and simultaneously prevent caspase activation by which they prevent the apoptotic process.[22] Anti-apoptotic Bcl-2 and Bcl-xL protein were the first known molecular targets of mitochondrial p53.[23] Interestingly, p53 can participate in apoptosis induction by

direct action on mitochondria. The apoptotic signals cause localization of p53 into the mitochondria to precede the release of cytochrome c and activation of procaspase-3.[24]

In the case of OLP, 92.9% Bcl-2 positive staining was found in inflammatory infiltration.[25] The overexpression of Bcl-2 was reported in oral dysplastic lesions and it was suggested that Bcl-2 plays an important role in apoptosis and oral tumorigenesis,[26,27] whereas the lack of expression of Bcl-2 in oral dysplasia was observed in other studies.[28,29] Importantly, dysregulation of the Bcl-2 gene expression in oral epithelial dysplasia may be an important causative factor for genetic aberrations in the progression of epithelial tumors.[9] In the present study higher expression of Bcl-2 and Bcl-xL are observed in all types of OLP lesions irrespective of OLP groups, indicating the apoptotic nature of OLP lesions. Comparatively, reduced expression of Bcl-2 and Bcl-xL in dysplastic lesions is an important indicator for a higher degree of apoptosis.
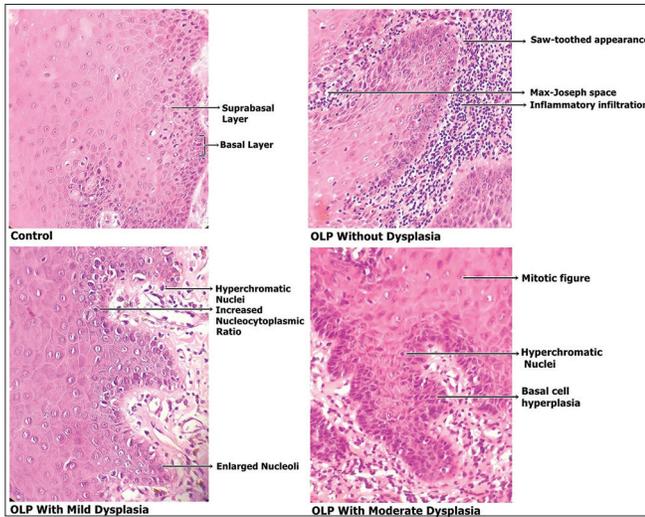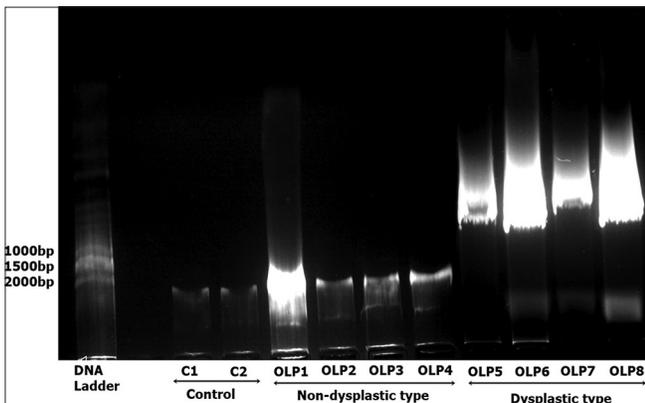
Different forms of oncogenic stress and DNA damage induce cell-cycle arrest, apoptosis or senescence which may activate the tumor-suppressor p53 to protect cells from malignant transformation.[30,31] This cellular accumulation of p53 protein can activate multiple other target genes which are required for tumor suppression.[21] The cyclin-dependent kinase inhibitor/p21 is one of them, whose promoter contains two p53-binding sites, where at least one of them is essential for p53 binding after DNA damage.[32] This binding inhibits apoptosis by causing cell cycle arrest and DNA repair. Later, the p21-arrested cells may undergo apoptosis by the activation of pro-apoptotic genes.[33]

Previous studies have shown that overexpression of p53 in OLP plays a regulatory role in apoptosis.[34] Elevated expression of p53 and p21 was observed in OLP patients when compared to normal but this did not differ from mild epithelial dysplasia indicating the precancerous potentiality of OLP lesions.[20,35] In the present study, mRNA expression of p53 and p21 were significantly elevated in both types of OLP patients compared to healthy individuals. Higher expression of p53 and p21 in dysplastic lesion compared with nondysplastic may play a critical role in apoptosis signaling as well as malignant transformation.

Most HSPs are rapidly overexpressed in response to cellular injuries including genotoxic stress. HSPs protect cells from apoptosis by preventing mitochondrial apoptosome formation and caspase activation.[36] HSPs promote tumorigenesis by

Figure 1: Histological images showing the presence of different characteristic features in nondysplastic and dysplastic lesions (H and E; ×40)

Figure 2: DNA fragmentation pattern in non-dysplastic and dysplastic OLP lesions compared with control
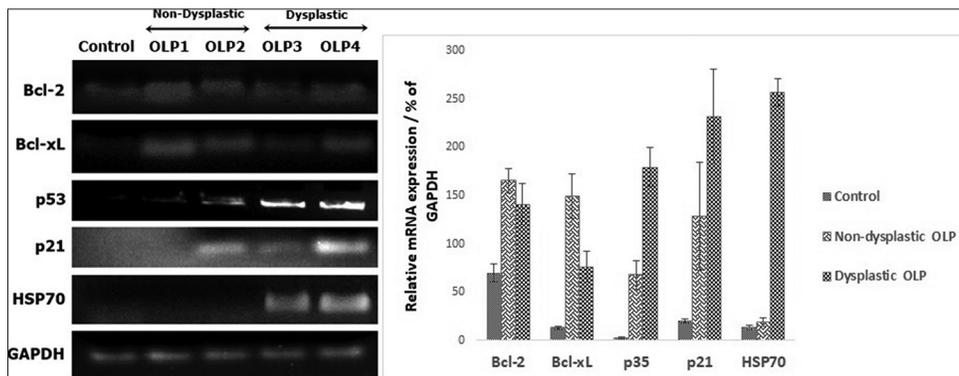
Figure 3: Comparative analysis of Bcl-2, Bcl-xL, p53, p21 and HSP70 gene expression in nondysplastic and dysplastic OLP lesion compared to control

suppressing apoptosis.[37] Increased expression of HSP70 is a marker for the presence of epithelial dysplasia or epithelial malignant transformation.[38,39] Upregulation of HSP70 was noted in premalignant lesions and oral squamous cell carcinoma.[40] Altered expression of HSP70 has been reported in OLP and oral leucoplakia, with the highest intensity in severe oral dysplasia which suggested that HSP70 may be an objective marker for the identification of epithelial dysplasia.[41,42] Similar results were found in our study, where HSP70 was overtly expressed in dysplastic lesions compared to nondysplastic type. This may be a prime indicator for higher cellular stress and the chance of malignant transformation in dysplastic OLP lesions compared to the nondysplastic type.

Alteration of multiple signaling pathways may cause deregulated apoptosis which is an important contributor for malignant transformation.[43] This is a mechanism of immune evasion which causes delayed apoptosis and prolongs inflammatory cell survival.[44] The detailed pathogenic mechanism of OLP is partially understood, most importantly the initial events in lesion formation and mechanism of keratinocytes apoptosis in OLP need to be addressed.[45] The comparative analysis of DNA fragmentation and expression of HSP70 in nondysplastic and dysplastic OLP lesions have not yet looked into, so this study may provide critical information about the pathogenesis mechanism.

Conclusion

Apoptosis is an important biological process involved in physiology and pathology. Nondysplastic OLP lesions have potency to transform into a dysplastic lesion and the dysplastic lesions have higher malignant potentiality but the detailed mechanism yet to be understood. In the present study, we have tried to evaluate the causative factors for the dysplastic and nondysplastic OLP lesion formation, which have an important role of pathogenesis mechanism. Intracellular stress signaling causes high DNA fragmentation which activates apoptotic pathways in OLP lesions. Elevated DNA fragmentation and reduced expression of Bcl-2 and Bcl-xL in the dysplastic lesion are indicators of the high rate of apoptosis when compared with nondysplastic type. Overexpression of p53, p21 and HSP70 in dysplastic OLP lesions have regulatory role in apoptosis signaling as well as in malignant transformation.

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Conflicts of interest

There are no conflicts of interest.

References

1. Lavanya N, Jayanthi P, Rao UK, Ranganathan K. Oral lichen planus: An update on pathogenesis and treatment. J Oral Maxillofac Pathol 2011;15:127-32.
2. Andreasen JO. Oral lichen planus. 1. A clinical evaluation of 115 cases. Oral Surg Oral Med Oral Pathol 1968;25:31-42.
3. Gorouhi F, Davari P, Fazel N. Cutaneus and mucosal lichen planus: A comprehensive rewiew of clinical subtypes, risk factors, diagnosis and prognosis. Sci World J 2014;2014:1-22.
4. Gupta S, Jawanda MK. Oral lichen planus: An update on etiology, pathogenesis, clinical presentation, diagnosis and management. Indian J Dermatol 2015;60:222-9.
5. Misra A, Rai S, Misra D. Functional role of apoptosis in oral diseases: An update. J Oral Maxillofac Pathol 2016;20:491-6.
6. Wong RS. Apoptosis in cancer: From pathogenesis to treatment. J Exp Clin Cancer Res 2011;30:87.
7. Gonzalez-Moles MA, Scully C, Gil-Montoya JA. Oral lichen planus: Controversies surrounding malignant transformation. Oral Dis 2008;14:229-43.
8. Shailaja G, Kumar JV, Baghirath PV, Kumar U, Ashalata G, Krishna AB. Estimation of malignant transformation rate in cases of oral epithelial dysplasia and lichen planus using immunohistochemical expression of Ki-67, p53, BCL-2, and BAX markers. Dent Res J (Isfahan) 2015;12:235-42.
9. Loro LL, Johannessen AC, Vintermyr OK. Decreased expression of bcl-2 in moderate and severe oral epithelia dysplasias. Oral Oncol 2002;38:691-8.
10. Vidya GD. Apoptosis in health and disease. Global Res Anal 2017;2:186-8.
11. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. Nat Rev Mol Cell Biol 2014;15:49-63.
12. Birchall MA, Schock E, Harmon BV, Gobe G. Apoptosis mitosis PCNA and Bcl2 in normal, leukoplakic and malignant epithelia of the human oral cavity: Prospective, *in-vivo* study. Oral Oncol 1997;33:419-25.
13. Arya R, Mallik M, Lakhotia SC. Heat shock genes – Integrating cell survival and death. J Biosci 2007;32:595-610.
14. Jiang B, Liang P, Deng G, Tu Z, Liu M, Xiao X. Increased stability of Bcl-2 in HSP70-mediated protection against apoptosis induced by oxidative stress. Cell Stress Chaperones 2011;16:143-52.
15. Rad M, Hashemipoor MA, Mojtahedi A, Zarei MR, Chamani G, Kakoei S, *et al*. Correlation between clinical and histopathologic diagnoses of oral lichen planus based on modified WHO diagnostic criteria. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:796-800.
16. Tampa M, Caruntu C, Mitran M, Mitran C, Sarbu I, Rusu LC, *et al*. Markers of oral lichen planus malignant transformation. Dis Markers 2018;2018:1959506.
17. Abdel-Latif AM, Abuel-Ela HA, El-Shourbagy SH. Increased caspase-3 and altered expression of apoptosis-associated proteins, Bcl-2 and Bax in lichen planus. Clin Exp Dermatol 2009;34:390-5.
18. Majtnerová P, Roušar T. An overview of apoptosis assays detecting DNA fragmentation. Mol Biol Rep 2018;45:1469-78.
19. Shailaja G, Kumar JV, Baghirath PV, Kumar U, Ashalata G, Krishna AB. Estimation of malignant transformation rate in cases of oral epithelial dysplasia and lichen planus using immunohistochemical expression of Ki-67, p53, BCL-2, and BAX markers. Dent Res J (Isfahan) 2015;12:235-42.
20. Sousa FA, Paradella TC, Carvalho YR, Rosa LE. Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia. J Oral Sci 2009;51:117-21.
21. Harris SL, Levine AJ. The p53 pathway: Positive and negative

feedback loops. Oncogene 2005;24:2899-908.

22. Gogvadze V, Orrenius S, Zhivotovsky B. Multiple pathways of cytochrome c release from mitochondria in apoptosis. Biochim Biophys Acta 2006;1757:639-47.

23. Ha JH, Shin JS, Yoon MK, Lee MS, He F, Bae KH, *et al*. Dual-site interactions of p53 protein transactivation domain with anti-apoptotic Bcl-2 family proteins reveal a highly convergent mechanism of divergent p53 pathways. J Biol Chem 2013;288:7387-98.

24. Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J Biol Chem 2000;275:16202-12.

25. Pigatti FM, Taveira LA, Soares CT. Immunohistochemical expression of Bcl-2 and Ki-67 in oral lichen planus and leukoplakia with different degrees of dysplasia. Int J Dermatol 2015;54:150-5.

26. Ravi D, Nalinakumari KR, Rajaram RS, Nair MK, Pillai MR. Expression of programmed cell death regulatory p53 and bcl-2 proteins in oral lesions. Cancer Lett 1996;105:139-46.

27. Yao L, Iwai M, Furuta I. Correlations of bcl-2 and p53 expression with the clinicopathological features in tongue squamous cell carcinomas. Oral Oncol 1999;35:56-62.

28. Schoelch ML, Le QT, Silverman S Jr, McMillan A, Dekker NP, Fu KK, *et al*. Apoptosis-associated proteins and the development of oral squamous cell carcinoma. Oral Oncol 1999;35:77-85.

29. McAlinden RL, Maxwell P, Napier S, Hamilton P, Cowan CG, Lundy FT, *et al*. Bcl-2 expression in sequential biopsies of potentially malignant oral mucosal lesions assessed by immunocytochemistry. Oral Dis 2000;6:318-26.

30. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307-10.

31. Georgakilas AG, Martin OA, Bonner WM. p21: A Two-Faced Genome Guardian. Trends Mol Med 2017;23:310-9.

32. Gartel AL, Radhakrishnan SK. Lost in transcription: p21 repression, mechanisms, and consequences. Cancer Res 2005;65:3980-5.

33. Abbas T, Dutta A. p21 in cancer: Intricate networks and multiple activities. Nat Rev Cancer 2009;9:400-14.

34. Oliveira Alves M, Balducci I, Rodarte Carvalho Y, Cabral L, Nunes F, Almeida J. Evaluation of the expression of p53, MDM2, and SUMO-1 in oral lichen planus. Oral Dis 2013;19:775-80.

35. Safadi RA, Al Jaber SZ, Hammad HM, Hamasha AA. Oral lichen planus shows higher expressions of tumor suppressor gene products of p53 and p21 compared to oral mucositis. An immunohistochemical study. Arch Oral Biol 2010;55:454-61.

36. Bivik C, Rosdahl I, Ollinger K. Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome c in human melanocytes. Carcinogenesis 2007;28:537-44.

37. Jäättelä M. Escaping cell death: Survival proteins in cancer. Exp Cell Res 1999;248:30-43.

38. Seoane JM, Varela-Centelles PI, Ramirez JR, Cameselle-Teijeiro J, Romero MA, Aguirre JM. Heat shock proteins (HSP70 and HSP27) as markers of epithelial dysplasia in oral leukoplakia. Am J Dermatopathol 2006;28:417-22.

39. Markopoulos AK, Deligianni E, Antoniades DZ. Heat shock protein 70 membrane expression in oral cancer: A possible new target in antineoplastic therapy? Chemotherapy 2009;55:211-4.

40. Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 1993;178:1391-6.

41. Seoane J, Ramírez JR, Romero MA, Varela-Centelles P, Garcia-Pola MJ. Expression of heat shock protein (HSP70) in oral lichen planus and non-dysplastic oral leucoplakia. Clin Otolaryngol Allied Sci 2004;29:191-6.

42. Tyagi N, Shetty DC, Urs AB. Altered expression of HSP70 in oral lichen planus. J Oral Maxillofac Pathol 2012;16:189-94.

43. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2016;35:495-516.

44. Bianchi SM, Dockrell DH, Renshaw SA, Sabroe I, Whyte MK. Granulocyte apoptosis in the pathogenesis and resolution of lung disease. Clin Sci (Lond) 2006;110:293-304.

45. Carrozzo M. Understanding the pathobiology of oral lichen planus. Curr Oral Health Rep 2014;1:173-9.