Original Article

Correlation of Bcl-2 and COX-2 Expression in Oral Lichen Planus

N. Taghavi [1], N. Mahdavi [2], M. Shahla [3].

[1]Member of Dental Research Center and Assistant Professor, Department of Oral and Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Oral and Maxillofacial Pathologyst
[3]Postgraduate Student, Department of Oral and Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background and Aim: Lichen planus is a common immune-mediated disease that is associated with an increased risk of malignant transformation in the oral cavity. Synchronous up-regulation of COX-2 and down-regulation of Bcl-2 have been demonstrated in some malignancies. The aim of this study was to assess the correlation between COX-2 and Bcl-2 expression and their role in dysplastic changes of oral lichen planus (OLP).

Materials and Methods: This study was performed on 47 paraffin blocks with the diagnosis of OLP and 16 blocks with the diagnosis of focal fibrous hyperplasia (control group). Immunohistochemical staining was performed using antibodies against COX-2 and Bcl-2. Spearman's rank correlation coefficient and Mann-Whitney test were used for data analysis.

Results: A significant correlation was observed between the intensity of sub-epithelial inflammation and the severity of basal cell layer degeneration ($p=0.048$). Significant up-regulation of Bcl-2 and COX-2 was detected in sub-epithelial inflammatory infiltration ($p<0.001$, $p=0.003$). The amount and intensity of Bcl-2 and COX-2 expression were significantly correlated in sub-epithelial lymphocytic infiltration ($p=0.013$, $p=0.019$)

Conclusion: Our findings indicated the effective role of Bcl-2 expression in decreasing the apoptosis in the inflammatory infiltrate unlike the epithelium. The significant correlation of the intensity of Bcl-2 expression in the epithelium and the sub-epithelial inflammatory infiltrate with COX-2 expression and also the correlation of the intensity of inflammation with the severity of basal layer hydropic degeneration may imply that these two markers can induce malignant transformation in the affected epithelium in an indirect manner by the continuation of inflammation and activation of carcinogenic mechanisms.

Key Words: Oral lichen planus, COX-2, Bcl-2

Corresponding author:
N. Mahdavi, Oral and Maxillofacial Pathologyst
n-mahdavi@tums.ac.ir

Received: 8 June 2013
Accepted: 7 Nov 2013

Journal of Islamic Dental Association of IRAN (JIDAI) Spring 2014 ;26, (2)

Introduction

Lichen planus is a chronic, immune-mediated disease of the skin and mucous membranes. It has a prevalence rate of 2% and mostly involves oral mucosa. Oral lichen planus (OLP) clinically manifests in reticular, plaque-like, atrophic, erosive and bullous forms. Reticular form is the most common type. Erosive and atrophic forms are associated with the risk of dysplastic changes and malignancy (squamous cell carcinoma) [1-3]. Based on the duration of follow-up, different studies have reported 0.65-6.5% risk of malignant transformation [4, 5]. However, some others believe that the risk of malignancy in OLP lesions is low [2, 6]. Several hypotheses have been suggested regarding the initiating factors (stimulants). Regardless of the nature of stimulant, it induces the migration of lymphocytes by the production of cytokines such as Tumor Necrosis Factor α (TNF-α), interleukin 1 (IL-1) and interferon α (IFN-α). Moreover, increased expression of adhesion molecules on the surface of lymphocytes and endothelium causes the migration of

lymphocytes. In this disease, lymphocytes attack the basal layer keratinocytes and induce their apoptosis [1].

COX2 enzyme is not present in healthy tissues. It is an inductive enzyme with a considerably increased production during pathologic conditions such as inflammation and malignancies [12]. Increased COX-2 expression has been reported in the stomach, pancreatic and lung cancer [7]. Its mechanism of action seems to be inhibition of apoptosisin tumoral cells. It acts independently of Bcl-2 in some tumors like lymphoma while it is Bcl-2-dependent in some others [8].

Bcl-2 is a member of anti-apoptotic Bcl-2 family. It maintains the integrity of the external mitochondrial membrane and inhibits apoptosis by direct attachment to the external mitochondrial membrane and inhibition of pro-apoptotic members of Bcl-2 family. Synchronousupregulation of Bcl-2 and Cox-2 has been shown in some malignancies such as colon cancer [9]. Considering the role of these two markers in apoptosis, dysplastic changes and malignancies as well as the mechanism of development of lichen planus and apoptosis of basal layer cells induced by the lymphocytes in this disease, this study was undertaken to assess the role of COX2 and Bcl-2 markers in dysplastic changes in OLP and evaluate the correlation between their expression in this disease.

Materials and Methods

This study was conducted on 47 paraffin blocks with the diagnosis of OLP (test group) and 16 control specimens with the diagnosis ofirritation fibromain the Pathology Department of Shahid Beheshti University, School of Dentistry. Specimens with incomplete files, indefinite diagnosis or inadequate tissue were excluded from the study. A questionnaire including demographic information of subjects and histological analysis by H&E and IHC staining was completed for each specimen.

In histopathological analysis, the specimens were separately evaluated for superficial keratosis (no keratosis, orthokeratosis, parakeratosis, ortho/ parakeratosis), morphology of the connective tissue-epithelium interface (papillary, flat), presence or absence of acanthosis, spongiosis, granulosis and lymphocytic exocytosis.

In terms of the degree of hydropic degeneration of basal layer, the specimens were divided into 3 groups of I (less than 25%), II (25-50%) and III (more than 50%). In terms of the intensity of subepithelial inflammation, the specimens were categorized into 3 groups of mild, moderate and severe.

In terms of dysplastic changes and malignancy, the specimens were categorized as no dysplasia, mild dysplasia, moderate dysplasia, severe dysplasia and squamous cell carcinoma (SCC).

For IHC analysis, specimens were fixed in 10% formalin, embedded in blocks and sectioned into 4µ slices by a microtome. Slides were stored at 37°C for 24h and 60°C for one hour to eliminate excess paraffin from the slide. Rehydration and deparaffinization were then performed by the immersion of specimens in 100% xylene and graded alcohol. Antigens were retrieved by the immersion of slides in TBS buffer and placing in a microwave (15min for Bcl-2 and 22min for COX2). Primary antibodies of Bcl-2 (monoclonal antibody clone 3.1, UK, Novocastra, ready to use) and COX2 (monoclonal mouse anti-human, clone 4H12, UK, Novocastra) were incubated at room temperature for one hour. For COX2, according to the manufacturers' recommendations, the antibody was diluted 1:50-1:100 using dilution solution.It was then rinsed in a container containing TBS with a pH of 7.6 for 5min and stored for one hour in an EnVision tube (Dako) and rinsed with TBS buffer. DAB was applied and then rinsed under running water and mounted after differential staining.

The percentage of cells stained for Bcl-2 marker was calculated by countingcells in 10 randomly selected fieldsat 200X magnification as follows:

0=0%,+=1%-10%, ++=11%-25%, +++=26%-50%, ++++=more than 50%

Also, the percentage of cells stained for COX2 marker was calculated similar to Bcl-2 as follows [11]:

0=0%, I=1%-19%, II=20%-49% and III=over 50%.

Ulcerative colitis and tonsillar specimens were used as the positive control for COX2 and Bcl-2, respectively. Endothelial staining was used as the internal control for COX2. The staining intensity of both markers was assessed in three levels of mild, moderate and severe.

It should be noted that the percentage of stained cells in basal and supra-basal layers and lymphocytic infiltrateswas separately calculated.

Data were analyzed using PASW software version 18. The correlation between the expression of COX2 and Bcl-2 markers with one another and also the severity of dysplasia and other histological parameters was assessed using Spearman's correlation coefficient. Mann Whitney test was applied to compare cases and controls. P<0.05 was considered statistically significant.

Results

The mean age of patients was 46.52±13.15 yrs. (range 21-68 yrs.); 30 lesions (68.2%) were in females and 14 (31.8%) in males. Evaluation of the location of lesions showed that the majority of lesions (33 cases, 75%) were located in the buccal mucosa. The least frequent site was the floor of the mouth with only one case (2.3%). Clinically, 27 lesions (64.3%) were erosive/atrophic, 13 lesions (31%) were reticular and 2 cases (4.8%) were bullous. The results of microscopic analysis are shown in Table 1. Histologically, the only significant correlation was found to be between the severity of inflammation and degree of hydropicde generation of the basal layer (p=0.048) (Table 2).

Table 1. The frequency distribution of specimens based on histological characteristics

Variable		Number (%)
Keratosis	Parakeratosis	32(%68/1)
	Orthokeratosis	8(%17)
	Para and Orthokeratosis	7(%14/9)
Acanthosis	No	15(%31/9)
	Yes	32(%68/1)
Granulosis	No	22(%46/8)
	Yes	25(%53/2)
Spongiosis	No	32(%68/1)
	Yes	15(%31/9)
Exocytosis	No	6(%12/8)
	Yes	41(%87/2)
Hydropicdegeneration	I (>25%)	5(%10/6)
	II (25-50%)	26(%55/3)
	III (>50%)	16(%34/0)
Epithelial detachment	No	24(%51/1)
	Yes	23(%48/9)
Epithelium-conn ective tissue interface	Papillary	32(%68/1)
	Flat	15(%31/9)
Severity of inflammation	Mild	3(%6/4)
	Moderate	24(%51/1)
	Severe	20(%42/6)
Dysplasia	No	27(%57/4)
	Mild	12(%35/5)
	Moderate	5(%10/6)
	Severe	2(%4/3)
	SCC	1(%2/1)

Immunohistochemically, the percentage and intensity of Bcl-2 and COX2 stainingof the epithelium and subepithelial inflammatory infiltrate are shown in Table 3 and Figures 1 and 2. The only significant difference in the expression of Bcl-2 and COX2 between the two groups of cases and controls was in inflammatory infiltrates (p=0.003, p<0.001) and expression of these two markers was not significantly different in the epithelium of case and control groups. Moreover, the expression of these two markers was not significantly associated with basal layer degeneration, severity of inflammation or dysplasia (Table 2).

Immunohistochemical analysis of the expression of Bcle-2 revealed no significant difference in the level and intensity of expression of Bcl-2 in the epithelium of cases and controls (Table 3). But the difference in subepithelial inflammatory infiltrates between the two groups in this respect was statistically significant (p=0.029, p<0.001).

As observed in Table 3, the level and intensity of the expression of COX2 in the epithelium, similar to Bcl-2, were not significantly different between the two groups of cases and controls. However, the difference in COX2 expression in subepithelial inflammatory infiltrates was significant between the two groups (p=0.003).

The expression of COX2 or Bcl-2 was not significantly associated with basal layer degeneration, severity of inflammation or dysplasia (Table 2).

A significant direct correlation was found between the expression of Bcl-2 and COX2 in subepithelial inflammatory infiltrates (p=0.013, p=0.019). In the epithelium, only the intensity of Bcl-2 and COX2 staining had a direct correlation (p=0.043). The intensity of expression of Bcl-2 in subepithelial inflammatory infiltrateshad a statistically significant association with the expression of COX2 in the basal (p=0.028) and supra-basal layers (p=0.021) and subepithelial inflammatory infiltrates (p=0.002) (Table 4).

Discussion

In lichen planus, lymphocytes attack the basal layer keratinocytes. The lymphocytic infiltratesare mainly comprised of T cells. T lymphocytes present in the epithelium next to the basal layer are usually active CD8+ cells [1]. It appears that these cells attach to the Intercellular Adhesion Molecule I (ICAM1) present on the surface of keratinocytes

Table 2. The correlation of hydropic degeneration, severity of inflammation and dysplasia with the level and intensity of expression of COX2 and Bcl-2 markers

---		Degree of dysplasia	Severity of inflammation	Hydropic degeneration
Hydropic degeneration		0/05	*0/048	
Severity of inflammation		0/26		0/048
Degree of dysplasia			0/26	0/550
Intens ity of Bcl-2 staining	Epithelium	0/325	0/662	0/145
	Inflammatory infiltrates	0/281	0/367	0/245
Percentage of cells stained for Bcl-2	Basal	0/912	0/163	0/198
	Supra-basal	0/298	0/557	0/269
	Inflammatory infiltrates	0/574	0/099	0/985
Intensity of COX2 staining	Epithelium	0/519	0/115	0/253
	Inflammatory infiltrates	0/816	0/341	0/761
Percentage of cells stained for COX2	Basal	0/860	0/362	0/666
	Supra-basal	0/304	0/288	0/862
	Inflammatory infiltrates	0/582	0/488	0/345

***Statistically significant correlation**

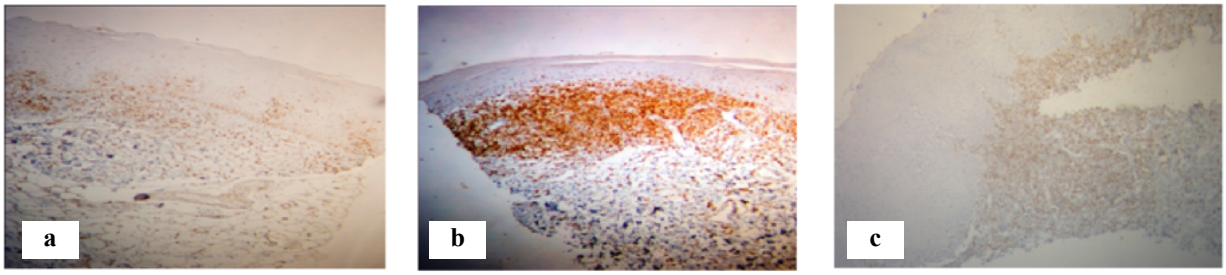

Figure 1. Bcl-2 staining
a. Moderate staining of subepithelial inflammatory infiltrates in non-dysplastic lichen planus
b. Severe staining of inflammatory infiltrates in mild dysplastic lichen planus (x100)
c. Mild staining of inflammatory infiltrates in lichen planus with malignant transformation (x100)

Figure 2. COX2 staining
a. Moderate staining of epithelium and inflammatory infiltrates in mild dysplastic lichen planus (x100)
b. Mild staining of epithelium and inflammatory infiltrates in lichen planus with severe dysplasia (x200)
c. Severe staining of inflammatory infiltrates in lichen planus with malignant transformation (x100)

Table 3. The level, intensity and correlation of expression of Bcl-2 and COX2 in the epithelium and lymphocytic infiltrates in the two groups of cases and controls

			Intensity of Bcl-2 staining				Percentage of cells stained for Bcl2					Intensity of COX2 staining				Percentage of cells stained for COX2			
-			Mild	Mod-erate	Severe	P.V	+	++	+++	++++	P.V	Mild	Mod-erate	Severe	P.V	I %1-19	II %20-49	III (<%50)	P.V
Epithelium	Control	Basal	7	2	1		5 %33/3	1 %6/7	2 %13/3	0 %0/0		3 %18/8	6 %37/5	7 %43/8		0 %0/0	2 %12/5	14 %87/5	
		Supra-basal	%43/8	%12/5	%6/3		4 %26/7	0 %0/0	0 %0/0	0 %0/0						4 %25	8 %50	4 %25	
	Case	Basal	8	12	4		15 %31/9	4 %8/5	0 %0/0	1 %2/1	0/345					1 %2/1	5 %10/6	39 %83/5	0/619
		Supra-basal	%17/0	%25/5	%8/5	0/789	10 %21/3	1 %2/1	0 %0/0	1 %2/1	1/000	6 %12/8	22 %46/8	16 %34	0/55 1	4 %8/5	21 %44/7	19 %40/4	0/259
Inflammatory infiltrates	Control		1 %12/5	9 %56/3	2 %12/5		8 %50/0	1 %6/3	3 %18/8	0 %0/0		1 %6/3	5 %31/3	3 %18/8		4 %25	2 %12/5	2 %12/5	
	Case		1 %2/1	32 %68/1	12 %24/5	*0/029	3 %4/6	7 %14/9	5 %10/6	30 %63/8	*0/000	0 %0/0	28 %59/6	10 %21/3	0/10 2	0 %0/0	10 %21/3	25 %53/2	*0/003

*Statistically significant correlation

Table 4. The correlation between Bcl-2 and COX2 markers

--		Percentage of cells stained for COX2			Intensity of COX2 staining	
		Inflammatory infiltrates	Supra-basal	Basal	Inflammatory infiltrates	Epithelium
Intensity of Bcl-2 staining	Epithelium	0/383	0/283	0/418	0/529	*0/043
	Inflammatory infiltrates	*0/002	*0/021	*0/028	*0/019	0/379
Percentage of cells stained for Bcl2	Basal	0/289	0/101	0/269	0/521	0/109
	Supra-basal	0/957	0/161	0/906	0/452	0/540
	Inflammatory infiltrates	*0/013	0/196	*0/021	0/059	0/190

via their surface receptors namely lymphocyte function-associated antigen I (LFA-I) and induce their apoptosis. Apoptosis is a key mechanism for prevention of malignancy. This mechanism is inactivated in different manners in malignant or premalignant lesions [12]. Different studies have investigated the level of apoptosis in OLP using TUNEL assay, Caspase 3 expression and light microscope [13].

Results of previous studies have shown that despite the higher rate of apoptosis compared to normal mucosa, level of apoptosis is usually low in OLP (10, 13). Some believe that this reduction is secondary to the up-regulation of Bcl-2 anti-apoptotic protein in the basal layer of OLP [14]. In our study, Bcl-2 was not expressed in 57.4% in basal and 74.5% in the supra-basal layers. Its expression was not significantly different in the two groups of cases and controls; which is in accord with previous studies indicating no or mild expression of Bcl-2 in the epithelium [10, 13-15]. Thus, it seems that the mechanism of decreased apoptosis of epithelium in OLP is probably independent of the Bcl-2 anti-apoptotic effects.

In this study, the expression of COX2 in the epithelium (basal and supra-basal layers) was not significantly different in various types of OLP and control group; which is in contrast to the results of Neppelberg and Renconen [16]. This difference may be due to the lower number of dysplastic specimens and cases with malignant transformations in our study or different staining techniquesin the two studies.

However, in our study, a statistically significant correlation was seen between the intensity of expression of Bcl-2 and COX2 in the epithelium. COX2 is located withinthe endoplasmic reticulum and nuclear membrane. Thus, it is detected by nuclear membrane staining [17-19]; its increased expression can be detected by cytoplasmic staining [20]. Generally, when assessing COX2 expression, cytoplasmic staining is indicative of positive expression; which per se is a sign of increased expression. In current techniques, the level of expression of COX2 depends on its intensity of expression. Using the intensity of expression of COX2 and Bcl-2 in the epithelium instead of their level of expression may be more accurate for the assess-

ment of these markers. Our literature search yielded no similar study evaluating the correlation between the intensity of expression of these two markers and future studies are required in this respect.

Furthermore, it seems that the protective mechanisms in the epithelium prevent the inductive effect of COX2 on Bcl2. Basconesshowed increased basal expression of p21 protein in OLP [13]. This protein is not expressed in the basal layer of normal epithelium and its expression in OLP indicates the stop of cell proliferation in the basal layer and DNA repair [21-23]. Other studies have shown that upregulation of p21 increases resistance to apoptosis [23]. However, although this effect is in accord with the anti-apoptotic effects of Bcl-2, a reverse correlation was seen between the expression of these two proteins andupregulation of p21 was associated with down-regulation of Bcl-2 [24, 25].

In contrast to epithelium, expression of COX2 and Bcl-2 was significantly different between the two groups of cases and controls in sub-epithelial inflammatory infiltrates and in 53.2% of cases,COX2 staining over 50% (score III) was seen in inflammatory cells. This rate was 63.8% for Bcl-2. These results are in accord with the findings of previous studies reporting low rate of apoptosis in OLP lymphocytic infiltrates [9, 26, 27] suggesting that decreased apoptosis in OLP lymphocytic infiltrates is due to the anti-apoptotic effects of Bcl-2 protein [27, 28]. In this study, level and intensity of expression of Bcl-2 in OLP lymphocytic infiltrates were significantly different from the control group (P=0.029 and P=0.000, respectively); which may be explained by both the upregulation of COX2 in inflammatory infiltrates of OLP compared to the control group and inductive effects of COX2 on Bcl-2.

On the other hand, considering the significant positive association between the intensity and level of expression of these markers, it appears that the increased resistance to apoptosis in inflammatory infiltrates (in contrast to epithelium) is due to the Bcl-2-dependent mechanisms and decreased apoptosis of inflammatory cells along with their increased proliferation and migration of new inflammatory cells are important factors responsible for persistent inflammation in OLP [10, 24]. Inflammatory infiltrates may be responsible for inhibition of apoptosis and increased proliferation of inflammatory cells in this disease via the inhibition of COX2, macrophage inhibition factor, RANTES and chemokine [10]. In our study, the correlation between the expression of Bcl-2 and COX2 and basal layer hydropicdegeneration (indicative of the severity of epithelial destruction) was not significant; whereas, a significant positive correlation was seen between the degree of basal layer hydropic degeneration and the severity of subepithelial inflammation. This issue may indicate that the intensity of inflammation is the main determinant of the severity of epithelial destruction. As mentioned earlier, increased cell proliferation and decreased apoptosis in the epithelium have been confirmed in OLP [10, 13]. Some researchers have suggested that these two mechanisms are activated in response to epithelial destruction and function as compensatory mechanisms to maintain the integrity of damaged epithelium. However, the consequence of this activity is the increased risk of dysplastic changes and sometimes malignancy in the epithelium [10, 13].

In this study, we failed to find a significant correlation between the severity of inflammation and dysplasia. However, these mechanisms are activated in response to epithelial destruction and level of epithelial destruction has a direct association with the severity of inflammation. Thus, it appears that a factor decreasing the severity of inflammation will indirectly decrease the risk of dysplastic changes in the epithelium. Therefore, although no significant correlation was noted between COX2 expression and the severity of epithelial destruction, administration of non-steroidal anti-inflammatory drugs (NSAIDs) may decrease the risk of dysplastic changes and malignancy in these lesions by inhibiting COX2 enzymes and decreasing the severity of inflammation. However, extensive investigations with higher number of dysplastic specimens are required to confirm this theory.

Conclusion

The results of this study showed that the level of expression of Bcl-2 in subepithelial inflammatory infiltrates of OLP, in contrast to epithelium, played an effective role in decreasing the level of apoptosis. Also, the intensity of Bcl-2 expression in the epithelium and inflammatory infiltrates was correlated with the level of expression of COX2. In our study, level of expression of COX2 was not corre-

lated with basal layer destruction and dysplastic changes in the epithelium. However, due to the direct association of the severity of inflammation and level of degenerationwith basal layer destruction, we may conclude that COX2 is indirectly correlated with epithelial destruction and activation of carcinogenesis mechanisms via continuation of inflammation.

References

1. Regezi JA, Sciubba JJ, Jordan RC. Oral Pathology. 5th ed. Missouri: Saunders; 2008, 90-5.
2. Neville B, Damm D, Allen C, Bouquqot E. Oral and Maxillofacial Pathology. 3rd ed. Philadelphia, Pensylvania: Elsevier; 2009, 782-8.
3. Rödström PO, Jontell M, Mattsson U, Holmberg E. Cancer and oral lichen planus in a Swedish population. Oral Oncol. 2004Feb; 40(2):131-8.
4. Eisen D. The clinical features, malignant potential and systemic associations of oral lichen planus: A study of 723 patients. J Am Acad Dermatol. 2002 Feb; 46(2):207-14.
5. Mignogna MD, Lo Russo L, Fedele S. Gingival involvement of oral lichen planus in a series of 700 patients. J Clin Periodontol. 2005Oct; 32(10):1029 -33.
6. Wang D, DuBois RN. Prostaglandins and cancer. Gut. 2006Jan; 55(1):115-122.
7. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998; 38:97-120.
8. Wun T, McKnight H, Tuscano JM. Increased cyclooxygenase-2 (COX-2): A potential role in the pathogenesis of lymphoma. Leuk Res. 2004 Feb; 28(2):179-90.
9. Liu XH, Yao S, Kirschenbaum A, Levine AC. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. Cancer Res. 1998 Oct; 58 (19):4245–9.
10. González-Moles MA, Bascones-Ilundain C, Gil Montoya JA, Ruiz-Avila I, Delgado-Rodríguez M, Bascones-Martínez A.Cell cycle regulating mechanisms in oral lichen planus: Molecular bases in epithelium predisposed to malignant transformation. Arch Oral Biol. 2006 Dec; 51(12):1093-103.
11. Pires I, Garcia A, Prada J, Queiroga FL. COX-1 and COX-2 Expression in canine cutaneous, Oral and ocular melanocytic tumors. J Comp Path. 2010 Aug-Oct; 143(2-3):142-9.
12. Wallace-Brodeur RR, Lowe SW. Clinical implications of p53 mutations. Cell Mol Life Sci. 1999Jan; 55(1):64-75.
13. Bascones C, Gonzalez-Moles MA, Esparza G, Bravo M, Acevedo A, Gil-Montoya JA. Apoptosis and cell cycle arrest in oral lichen planus Hypothesis on their possible influence on its malignant transformation. Arch Oral Biol. 2005Oct; 50(10): 873-81.
14. Tanda N, Mori S, Saito K, Ikawa K, Sakamoto S. Expression of apoptotic signaling proteins in leukoplakia and oral lichen planus: quantitative and topographical studies. J Oral Pathol Med. 2000 Sept; 29:385-93.
15. Sklavounou-Andrikopoulou A, Chrysomali E, Iakovou M, Garinis GA, Karameris A. Elevated serum levels of the apoptosis related molecules TNF-alpha, Fas/Apo-1 and Bcl-2 in oral lichen planus. J Oral Pathol Med. 2004Aug; 33(7):386-90.
16. Neppelberg E, Johannessen AC. DNA content, Cyclooxygenase-2 expression and loss of E-cadherin expression do not predict risk of malignant transformation in oral lichen planus. Eur Arch Otorhinolaryngol .2007Oct; 264 (10):1223-30.
17. Hla T, Bishop-Bailey D, Liu CH, Schaefers HJ, Trifan OC. Cyclooxygenase-1 and -2 isoenzymes. Int J Biochem Cell Biol. 1999; 31:551-7.
18. Tatsuguchi A, Sakamoto C, Wada K, Akamatsu T, Tsukui T, Miyake K, Futagami S. Localisation of cyclooxygenase 1 and cyclooxygenase 2 in Helicobacter pylori related gastritis and gastric ulcer tissues in humans. Gut. 2000Jun; 46(6):782-9.
19. Hashimoto Y, Kondo Y, Kimura G, Matsuzawa I, Sato S, Ishizaki M, Imura N, et al. Cyclooxygenase-2 expression and relationship to tumour progression in human renal cell carcinoma. Histopathology. 2004Apr; 44(4):353-9.
20. Wang W, Bergh A, Damber JE. Cyclooxygenase-2 expression correlates with local chronic infammation and tumor neovascularization in human prostate cancer. Clin Cancer Res. 2005May; 11(9):3250-6.
21. Poluha W, Poluha DK, Chang B, Crosbie NE, Schonhoff CM, Kilpatrick DL, et al. The cyclin-dependent kinase inhibitor p21 (WAF1) is required

for survival of differentiating neuroblastoma cells. Mol Cell Biol. 1996Apr; 16(4):1335-41.
22. Sheikh MS, Li XS, Chen JC, Shao ZM, Ordonez JV, Fontana JA. Mechanisms of regulation of waf1/Cip 1 gene expression in human breast carcinoma: Role of p53-dependent and independent signal transduction pathways. Oncogene. 1994 Dec; 9(12):3407-15.
23. Wang J, Walsh K. Resistance to apoptosis conferred by Cdk inhibitors during myocyte differentiation. Science. 1996 Jul; 273(5273):359-61.
24. Bukholm IK, Nesland JM, Kåresen R, Jacobsen U, Børresen-Dale AL. Interaction between bcl-2 and p21 (WAF1/CIP1) in breast carcinomas with wild-type p53. Int J Cancer. 1997Sept; 73(1):38-41.
25. Mańdziuk S, Dudzisz-Sledź M, Korszeń-Pilecka I, Milanowski J, Wojcierowski J, Korobowicz E. Expression of p21 and bcl-2 proteins in paraffin-embedded preparations of non-small cell lung cancer in stage IIIA after Etoposide and Cisplatin induced chemotherapy. Ann Univ Mariae Curie Sklodowska Med. 2003; 58(1):149-53.
26. Dekker NP, Lozada-Nur F, Lagenaur LA, MacPhail LA, Bloom CY, Regezi JA. Apoptosis-associated markers in oral lichen planus. J Oral Pathol Med. 1997Apr; 26(4):170-5.
27. Neppelberg E, Johannessen AC, Jonsson R. Apoptosis in oral lichen planus. Eur J Oral Sci. 2001Oct; 109(5):361-4.
28. Hirota M, Ito T, Okudela K, Kawabe R, Yazawa T, Hayashi H. Cell proliferation activity and the expression of cell cycle regulatory proteins in oral lichen planus. J Oral Pathol Med. 2002Apr; 31(4): 204-12.
29. Sugerman PB, Savage NW, Walsh LJ, Seymour GJ. Disease mechanisms in oral lichen planus. A possible role for autoimmunity. Australas J Dermatol. 1993; 34(2):63-9.