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Apoptosis In Oral Lichen Planus: Immunohistochemical Expression of Bcl-2 , P53 and Fas Molecules

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Abstract

Background: Apoptotic cell death may be a contributory cause of basal and parabasal keratinocytic destruction in oral lichen planus (OLP). Therefore, the aim of this study was to evaluate OLP specimens for apoptosis-regulating proteins; P53 & Fas as pro-apoptotic positive regulators and Bcl-2 as anti-apoptotic negative regulator, in comparison to the normal oral mucosa.

Material and Methods: The study was performed on ten histologically diagnosed lichen planus (group 1) together with ten oral mucosa biopsies taken from normal healthy subjects as controls (group 2).

Results: We found that bcl-2 protein was weakly expressed in 3/10; 30% of group 1 cases in a little number of basal & suprabasal keratinocytes although the melanocytes, subepithelial lymphoid cells and C.T. spindle & dendritic cells were intensely bcl-2-positive in all group 1 cases. A weak bcl-2 expression was detected in a slightly more number basal and suprabasal keratinocytes in 2/10; 20% of group 2 cases. However, the melanocytes in all group 2 cases were less intensely bcl-2-positive than those of group 1. The mean percentages of bcl-2-positive kerainocytic areas were statistically insignificant between both groups. Conversely, a moderate P53-positive staining was displayed in basal and suprabasal keratinocytes in all group 1 and 2 cases (100%) being much more numerous in basal than in suprabasal keratinocytes. The mean percentages of P53-positive keratinocytic areas were significantly higher in group 1 than in group 2. The subepithelial lymphoid tissue showed an occasional moderately to intensely stained P53-positive lymphocytes in all group 1 cases. Moreover, all both groups cases (100%) expressed weakly-to-moderately stained Fas-positive basal and suprabasal keratinocytes being much more numerous in basal than in suprabasal keratinocytes. The mean percentages of Fas-positive keratinocytic areas were significantly higher in group1 than in group 2. The subepithelial lymphoid tissue revealed weakly stained abundant Fas-positive mononuclear inflammatory cells either evenly distributed throughout the infiltrate or focally in some areas of the infiltrate as clusters of positive cells. The subepithelial Fas-Positive C.T. cells in group 1 were similar in their distribution to those in group 2 but more intense. The superficial keratinocytes in both groups were negatively immuno-reactive for any of these proteins.

Conclusion: Our study qualitatively evidenced the operative role of Bcl-2, P53 and Fas in OLP. In addition, these proteins may be involved in maintenance of the OLP lymphoid infiltrate and possibly in keratinocyte apoptosis. Moreover, this study can give a better understanding of apoptosis in OLP and hopefully may contribute in its future therapy.

Key words: Apoptosis – Bcl-2 protein – P53 protein – Fas molecule – Immunohistochemistry – Oral lichen planus.

Introduction

Lichen planus (LP) is a chronic inflammatory mucocutaneous disease. Clinical and immunohistochemical studies strongly support an immunologic basis for this disease *(Porter et el., 1997)*. The oral form is a persistent lymphocyte-mediated immunologic disorder in which basal & parabasal keratinocytes appear to be the targets of T- lymphocytes. Focal release of cytokines is thought to mediate recruitment and retension of lymphocytes (especially CD4 and CD8 cells) as well as death of basal and parabasal keratinocytes *(Becker and Schuppan, 1995; Regezi et al., 1996 & Sugermann et al., 1996)* which is a prominent morphologic feature of that disease *(Dekker et al., 1997)*.

Apoptosis is a term derived from apo (=away) and ptosis (=to fall over) and literally means falling off of petals from a flower or of leaves from a tree. This term was first coined by Kerr *et al.* in 1972 to describe programmed cell death. Apoptotic bodies, that are membrane-bound structures containing fragments of the nucleus and cellular organelles, may be phagocytosed by the cells; not known normally to be phagocytic in function such as keratinocytes *(Patki, 2002)*. The cells die by two mechanisms; necrosis and apoptosis. Necrosis occurs in various disease processes as a passive event while apoptosis is an active one which involves synthesis of RNA and proteins by the dying cell. Normally, apoptosis eliminate unwanted, senescent and damaged cells without harmful effects on the surrounding healthy cells. Defective or excessive apoptosis is involved in many disease processes *(Gibson, 2001)*. Defective apoptosis may be involved in autoimmune disorders while excessive process is likely to be a factor in neurodegenerative diseases such as Alzheimer's disease, Parkinsonism and amyotrophic lateral sclerosis *(Barinaga, 1998 & Gibson, 2001)*. Several dermatological disorders like toxic epidermal necrolysis (TEN), lichen planus and lichenoid reactions are known to occur due to apoptosis of cells either on a limited or massive scale *(Patki, 2002)*. Genetic alterations causing impeded apoptosis at a cellular level are defined in many immunologic diseases, lympho-proliferative diseases and some epithelial tumors and contribute significantly to the pathogenesis *(Miret et al., 1999; Senturk et al., 2001 & Yildiz et al., 2003)*.

Several gene-encoded proteins that may, by site and cell specificity, have inhibitory or stimulatory effects on apoptosis *(Hirota et al., 2002)*. Some of the well-recognized apoptosis-associated proteins include the Bcl-2 family (Bcl-2, Bax, Bcl-x), the Fas/Fas-ligand pair and P53. These proteins have been identified immunohistochemically in keratinocytes from inflammatory skin biopsies *(Rodrigues et al., 1995; Wrone et al., 1995; Dekker et al., 1997 & Hirota et al., 2002)*.

Bcl-2, which encodes a 25-KDa mitochondrial protein, is a member of Bcl-gene family and a proto-oncogene that protects cells from apoptosis appearing to be important to some cells than others *(Senturk et al., 2001 & Bal et al., 2008)*. It is highly expressed by melanocytes but weakly expressed by cutaneous keratinocytes *(Rodrigues et al., 1995; Wrone et al., 1995 & Dekker et al., 1997)*.

P53 protein which arrests the cell cycle, presumably to mediate DNA repair, may also interact with apoptosis-associated proteins to induce apoptosis if DNA damage is extensive *(Miyashita et al., 1994 & Schifter et al., 1998)*. The triggers that lead to the activation of this apoptotic mechanism include ligand binding to growth factors and cytokine receptors, exogenous stimuli (toxic agents, cellular damage) and viral infections *(Dekker et al., 1997)*. Mutations in the P53 tumor suppressor gene are the most common molecular defects detected in human malignancies including squamous cell carcinoma of the oral cavity. Significantly, carcinogenesis of the oral mucosa *(Raybaud et al., 1996 & Ogden and Hall, 1997)*. P53 mutations may result in formation of defective, highly stabilized protein with an increased half-life in tissues compared to the twenty minutes for the wild-type protein. This is the basis for the use of immunohistochemistry technique to detect

either P53 types *(Cox and Walker, 1996 & Schifter et al., 1998)*.

Fas receptor (CD95), one of the death receptors triggering the so-called caspase cascade, is a transmembrane protein of the tumor necrosis factor (TNF)-receptor family *(Thornberry and Lazebnik, 1998 & Patki, 2002)* that can activate a cell death signal when bound to a TNF-related cytokine; Fas-ligand *(Dekker et al., 1997)*. This receptor is an apoptosis-inducing receptor on the cell surface expressed by activated T- and B-lymphocytes as well as by rapidly proliferating cells *(Tanaka et al., 1995 & Neppelberg et al., 2001)*. In addition, this receptor has been demonstrated on the epidermal basal and parabasal keratinocytes in lymphocyte-mediated diseases including lichenoid drug reaction, lichen planus, erythema multiforme and contact dermatitis *(Sayama et al., 1994; Neppelberg et al., 2001 & Patki, 2002)*. Fas-L binds to Fas R leading to transducting signal that finally causes apoptosis via a caspase-dependant pathway. Moreover, Fas system is involved in activation-induced suicide of T-lymphocytes and down-regulation of the immune reaction *(Enari et al., 1998; Neppelgerg et al., 2001 & Sugermann et al., 2002)*.

The aim of this work is to utilize immunohistochemical technique in qualitative and quantitative expression of Bcl-2, P53 and Fas proteins as well as to investigate their role in apoptosis & maintaining the balance against or towards the cell death, in lesional OLP tissue compared to the normal mucosa.

Material and Methods

The study was performed on two groups **(Table 1)**:

*Group 1: Included ten selected OLP cases.

*Group 2: Included ten normal buccal mucosal biopsies taken as controls.

The group 1 cases were received at Department of Dermatology while the group 2 cases were taken from oral surgical procedures at Department of Dentooral Surgery at Al-Azhar University Hospitals with consent during the period from May 2006 till May 2007. The group 1 patients had a mean age of 50.8 ± 2.3 (range 28-65 years); three of whom were males (30%) while seven were females (70%) while the group 2 subjects had a mean age of 51.7 ± 1.9 (range 35-62 years); four of whom were males (40%) & six were females (60%). Clinically, two of group 1 patients (2/10, 20%) complained of a slight irritation while the remainings (8/10, 80%) were asymptomatic. Five (5/10, 50%) had a reticular form of OLP, one of which also had plaque lesions and another one had, as well, cutaneous lesions. The remaining (5/10, 50%) had an atrophic form **(Table 2)**.

All samples were fixed in 10% formalin and embedded in paraffin. The sections were cut at 6 µm and subjected to the following techniques:

1)Hematoxylin and Eosin (Hx & E) staining:

The staining was done to explore the morphologic appearance of the oral mucosa & lichen planus and to ensure the integrity of the specimens before the immunoperoxidase technique.

2)The immunoperoxidase (IP) technique:

Paraffin sections were downed to water. The endogenous peroxidase activity was blocked by incubating the sections in hydrogen peroxidase for 10 minutes & washed twice in phosphate buffered saline (PBS). Enzyme digestion was performed by incubating the sections in 0.1% trypsin solution at 37°C for 10 minutes followed by 4 changes in PBS. To block nonspecific background staining, sections were incubated in the "Ultra V Block" for 5 minutes at room temperature followed by rinsing in PBS. The sections were incubated in the primary monoclonal antibodies; anti-Bcl-2 (Clone 124, M0887, Dako) in a dilution of 1:100, anti-P53 (Clone DO-7, Novocastra) in a dilution of 1:200 and anti-Fas (Clone CH-11, IgM, Immunotech) in a dolution of 1:1000. The sections were washed 4 times in PBS, then, incubated in the Biotinylated Goat Anti-polyvalent solution for 10 minutes at room temperature followed by 4 changes in PBS. The streptavidin peroxidase was applied for 10 minutes at room temperature, then, the sections were washed 4 times in PBS. Finally, the sections were incubated with the chromogen substrate for 10 minutes,

counterstained with hematoxylin and coversliped. Normal mouse sera containing mixed immunoglobulins at a concentration approximating primary antibodies were used as negative controls. Immunohistochemically stained sections were graded subjectively as negative, weak, moderate or intense staining.

*** Immunoreactivity:**

Positive Bcl-2 reaction appeared as a brownish cytoplasmic staining while positive P53 signals appeared as a brownish nuclear and occasional cytoplasmic staining. Moreover, Fas immunopositivity was disclosed either as a brownish membranous, nuclear or small spots within the cell.

*** Quantitative analysis:**

For quantitative assessment, computerized image analysis was carried out. The mean percentage of positive keratinocytic area in relation to the total epidermal area was calculated using Image Pro Plus V4.51 *(Media Cybernetics Inc. 2002)*. Positive melanocytes, Langerhans cells as well as the inflammatory cells were excluded from the quantitative analysis but were qualitatively evaluated regarding the intensity of staining and the pattern of distribution in subepithelial infiltrate.

*** Statistical analysis:**

This was carried out according to the standard statistical procedures and student (T) test to estimate the significance of results using Microsoft Excel XP 2002.

Results

Morphological appearance:

The routinely stained sections revealed basic histological criteria **(Fig.1)** including hyperkeratosis, basal layer vacuolization & liquefaction as well as effacement of the epithelial-connective tissue interface with a band of intense lymphocytic infiltration.

Molecular Expression:

Bcl-2 was expressed weakly, in three out of ten (3/10, 30%) group 1 cases, in a little number of basal & suprabasal keratinocytes **(Fig.2)** although the melanocytes, many subepithelial lymphocytes and macrophages as well as C.T. spindle and dendritic cells were intensely stained for Bcl-2 protein in all group 1 cases.

This protein also was weakly detected in two out of ten (2/10, 20%) group 2 cases, in a slightly more number of basal and suprabasal keratinocytes than in group 1 while the melanocytes in all group 2 cases were less intensely Bcl-2 positive than those of group 1 **(Fig.3)**. the mean percentage of Bcl-2-positie basal and suprabasal keratinocytic areas were insignificantly higher in group 2 than in group 1 cases (P=0.935; **Table 3**). The superficial keratinocytes in both groups didn't stain for Bcl-2 protein.

Conversely, all group 1 cases (100%, **Fig.4)** showed a moderate P53-positive immunostaining in both basal & suprabasal keratinocytes being more numerous in basal than in suprabasal cells. The subepithelial lymphoid infiltrate displayed an occasional moderately to intensely stained P53-positive lymphocytes in all OLP cases. In addition, all group 2 cases (100%, **Fig.5)** revealed less moderately stained P534-positive basal and suprabasal keratinocytes than those in group 1 cases although more numerous P53-positive keratinocytes were found in basal than in suprabasal layer of control group cases. The mean percentages of P53-positive basal and suprabasal keratinocytic areas were significantly higher in group 1 than in group 2 (P=0.00027, **Table 3**). The superficial keratinocytes in all both groups cases were 53-negative.

Regarding Fas molecule, all group 1 cases (100%, **Fig.6)** expressed Fas-positive basal and suprabasal keratinocytes being much more numerous in the former cells. The subepithelial lymphoid tissue showed abundant weakly stained Fas-positive mononuclear cells either, most often, evenly distributed throughout the inflammatory infiltrate or occasionally focally distributed in the infiltrate as clusters of positive cells. The basal and suprabasal Fas-positive keratinocytes were weakly stained in the areas where the infiltrate was found and moderately stained in areas lacking the inflammatory infiltrate. Moreover, all group 2 cases (100%, **Fig.7)** displayed less numerous weakly to moderately stained Fas-positive

basal and suprabasal keratinocytes than those in group 1. In addition, the positive cells were more abundant in group 2 basal layer than in its suprabasal one. The subepithelial Fas-positive C .T. cells had a similar distribution in both groups despite of being more intense in group 1 than in group 2. The mean percentages of Fas-positive basal and suprabasal keratinocytic areas were significantly greater in group 1 than in group 2 (P=0.00031, **Table 3**). The superficial keratinocytes in either group didn't stain with anti-Fas antibody.

Table 1: Histopathological diagnosis of the studied cases (n=20):

Diagnosis	No;of cases	%
• Group 1: Oral lichen planus (OLP)	10	50
• Group 2: Normal oral mucosa (Controls)	10	50
Total	**20**	**100**

Table 2: Clinical findings of the studied cases (n=20):

Clinical Findings	Group 1	Group 2
• **Age (Years):**		
Range	28 – 65	35 – 62
Mean	50.8 ± 2.3	51.7 ± 1.9
• **Sex:**		
Male	3 (30%)	4 (40%)
Female	7 (70%)	6 (60%)
• **Symptoms:**		
Slight irritation	2 (20%)	
Asymptomatic	8 (80%)	
• **Clinical forms:**		
Reticular only	3 (30%)	
Reticular and Plaque	1 (10%)	
Reticular with cutaneous lesions	1 (10%)	
Atrophic only	5 (50%)	

Table 3: Immunohistochemical positivity in both studied groups (n=20):

I H C	Group 1			Group 2			P value
	B	S	Overall	B	S	Overall	
Bcl-2 positivity··	0.091 ± 0.007	0.08 ± 0.002	0.118 ± 0.008	0.315 ± 0.009	0.072 ± 0.004	0.401 ± 0.02	0.935*
P53 positivity··	17.64 ± 5.26	4.35 ± 2.23	19.1 ± 5.98	8.31 ± 3.27	2.28 ± 1.57	9.7 ± 2.39	0.00027·
Fas positivity··	19.31 ± 4.85	3.62 ± 1.95	21.14 ± 9.74	6.21 ± 2.18	1.51 ± 0.98	7.42 ± 2.09	0.00031·

B=Basal ; S = Suprabasal ; IHC = Immunohistochemistry
* = Non significant P value ; · = Significant P value

·· The immunopositivity is expressed as mean percentage of positive keratinocytic area in relation to the total epidermal area

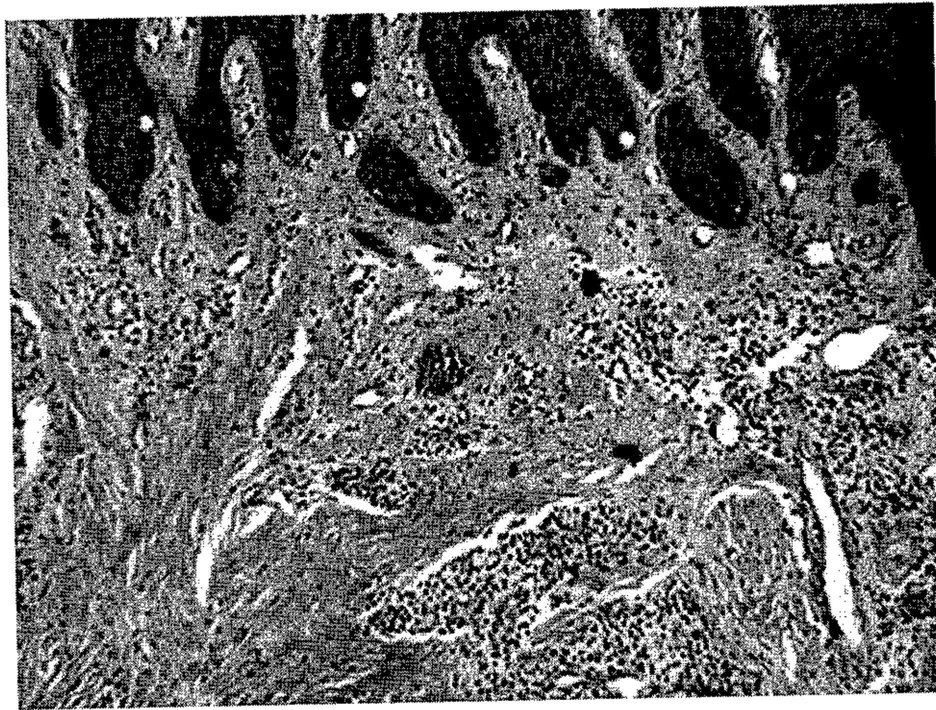

Fig . 1 : A case of oral lichen planus (OLP) showing hyperkeratosis , basal layer vacuolization as well as effacement of the epithelial – C. T . interface together with a band of intense lymphocytic infiltration .

(H x & E x 100)

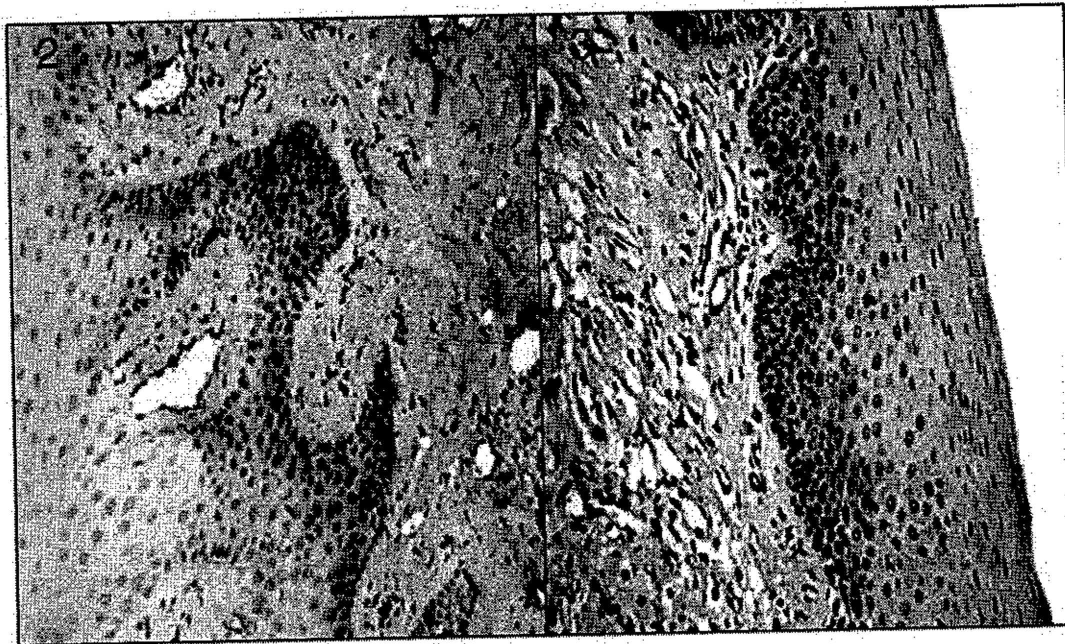

Fig . 2 : A case of OLP showing a weak expression of bcl-2 (brownish cytoplasmic staining) in basal and suprabasal keratinocytes (Immunoperoxidase [IP] X 200) . Fig .3 :A case of oral normal mucosa showing a weak bcl-2 cytoplasmic staining in basal & suprabasal keratinocytes (IP x 200).

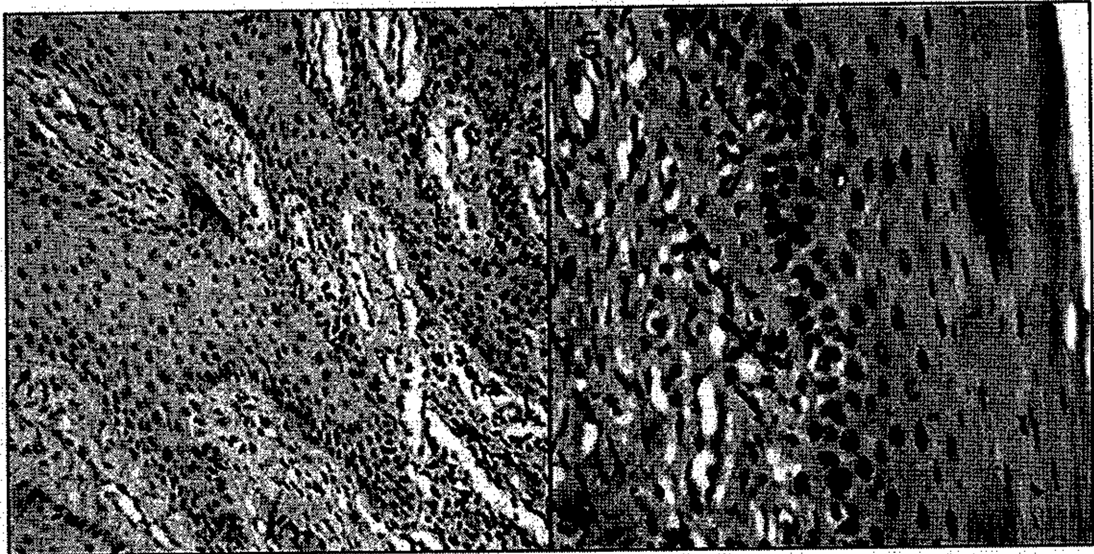

Fig. 4 : A case of OLP showing a moderately p53 – stained basal and suprabasal keratinocytes (brownish nuclear staining ; IP x 200) . Fig .5 : A case of oral normal mucosa revealed a moderately positive p53 – stained basal & suprabasal keratinocytes (IP x 400) .

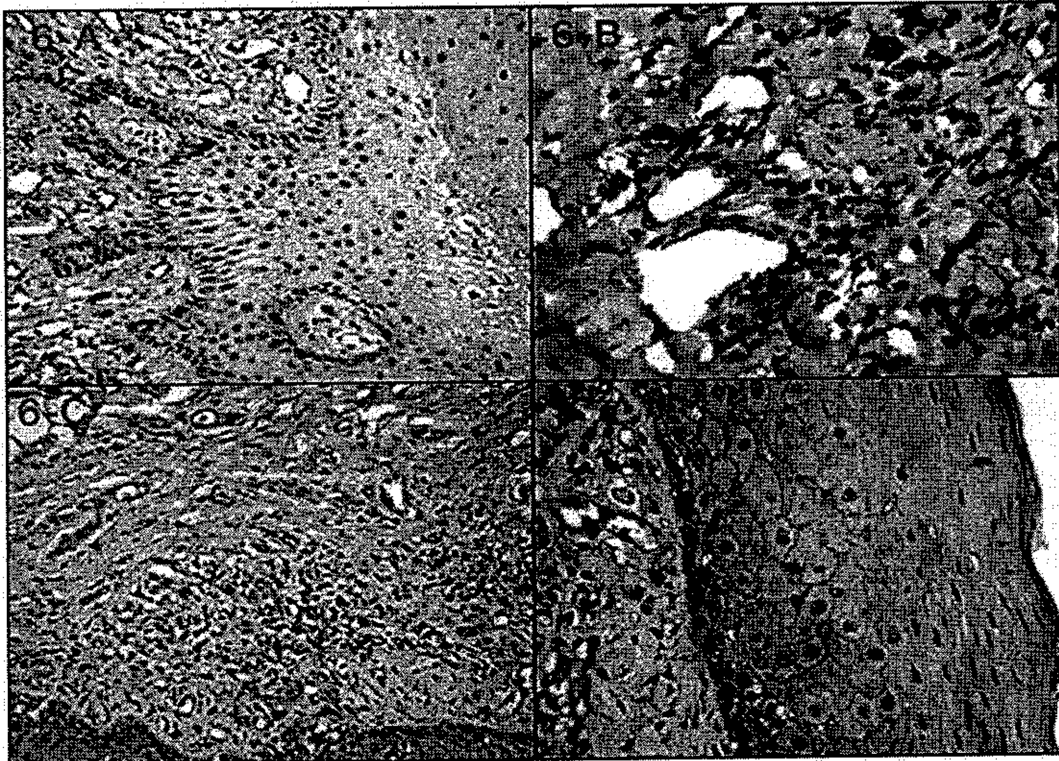

Fig .6 : A case of OLP showing numerous intensely Fas – positive basal and suprabasal keratinocytes (brownish nuclear staining ; 6A x 400) . 6B showed an abundant weakly to moderately stained subepithelial mononuclear cells focally (as clusters) distributed in the inflammatory infiltrate (x 400) . 6C displayed even distribution of the Fas – positive mononuclear cells throughout in the infiltrate (x 200) . Fig .7 : A case of oral normal nucosa displaying less number of weakly to moderately stained

Fas – positive (membranous reaction) basal & suprabasal keratinocytes than in Fig 6. (IP x 400)

Discussion

Lichen planus is one of recurrent chronic inflammatory dermatoses *(Yildiz et al., 2003)*. The oral type of that disease is characterized by epidermal cell injury and colloid body or apoptotic cell formation due to ultrastructural chromatin condensation at the nuclear membrane, cell shrinkage and compaction of the organelles *(Patki, 2002)*. This process is known to be mediated by the action/interaction of many proteins and cell surface receptors *(Dekker et al., 1997)*.

Our study revealed weak bcl-2 expression in a little number of basal and suprabasal keratinocytes in 30% of group 1 cases and in 20% of group 2 cases. Although the bcl-2-positive keratinocytes in group 2 were slightly more than those in group 1, there was no definite differences in the mean percentages of bcl-2 positive keratinocytic areas between both groups. In addition, all group 1 cases showed intensely bcl-2 immunostaining for melanocytes, many subepithelial mononuclear inflammatory cells as well as the subepithelial C.T. cells while the bcl-2-positive melanocytes detected in all group 2 cases were less intense in staining than those of group 1. These results were in concordance with those observed by Dekker *et al.* 1997, Boyd *et al.*, 1997; Neppelbeg *et al.* 2001 & Bal *et al.* 2008 who mentioned that Bcl-2 expression in the subepithelial inlfmmatory infiltrate of OLP results not only in an increased survival but also in continuous and regular cytokine secretion which may explain the chronicity and recurrence of this disease. Moreover, the anitapoptotic changes in the subepithelial inflammatory cells may be active in OLP pathogenesis either primarily or secondarily. Yildiz *et al.* (2003), Karatsaidis *et al.* (2004) and Bal *et al.* (2008) found Bcl-2 over-expression in the dermal $CD3^+$ T-lymphocytes and reported that the autoreactive T-cells may be valid both in psoriasis vulgaris and lichen planus. Thus, these dermatoses may be autoimmunity-related diseases although they are not purely autoimmune.

Insignificant difference in the keratinocytic Bcl-2 immunopositivity between OLP and normal nucosa detected in our and Sklavounou *et al.* (2000) studies explains that this protein does not seem to be greatly involved in the epithelial changes of that disease.

Khan *et al.* (2003) & Sklavounou *et al.* (2004) noticed a possible association of Bcl-2 serum level downregulation found in the atrophic-erosive OLP with the susceptibility of T-cells apoptosis in the subepithelial infiltrate. They, as well, mentioned that inadequate immunosuppression may promote the hyperactive immune responses in OLP. Furthermore, a possible selective apoptosis of the putative immunosuppressive T-cells in that disease may be associated with the promotion of the disease activity.

We displayed a moderately P53 immunostained basal and suprabasal keratinocytes in all group 1 & 2 cases. The P53-positive keratinocytes were more numerous in the basal than in the suprabasal layers in both groups. A significantly higher mean percentages of basal and suprabasal keratinocytic areas were found in group 1 compared to the group 2. Also, the subepithelial inflammatory infiltrate of group 1 cases showed an occasional moderately to intensely P53-stained lymphocytes. These findings coincided with those of Dekker et al., 1997 and Valente *et al.*, 2001 who mentioned that the P53 staining, encountered in OLP, is due to overexpression of a wild-type more than the mutant type of this protein since the malignant transformation is a rare event in this disease. Moreover, even if dysplasia is not related per se to a certain progressive neoplastic transformation, it is associated with an increase of P53-positive cells whose percentage was significantly greater in the transformed than in the non-transformed cases. Dekker *et al.* (1997) stated that P53 overexpression in OLP is

believed to have a protective effect by arresting the cell cycle for DNA repair that had been mediated by the lymphophagocytic infiltrate found in that disease. In instances of severe injury, it may activate apoptosis so that the defective cell is eliminated. This explains the fact based upon the typical basal position of P53-positive keratinocytes in OLP although, Valente *et al.* (2001) detected a number of P53-positive cells in cases of carcinoma, transformed from OLP without preceding dysplastic changes, beyond the basal layer and this is not consistent with a cross-reaction with the wild form of P53.

Shifter *et al.* (1998) studied a series of OLPs by both P53 immunostaining and the single strand confirmational polymorphism (SSCP) technique for P53 gene mutations and found a discrepancy between the two methods as ten strongly P53-positive cases didn't show mutations and concluded that P53 immunohistochemical overexpression may imply a physiological and antimutagenesis protective response in the epithelium in a hyperproliferative state. This may be a factor in the low incidence of carcinoma associated with OLP.

This study revealed weakly to moderately Fas-immunostained keratinocytes in the basal and suprabasal layers of all both groups cases being much more numerous in basal than in suprabasal layer. The mean percentages of Fas-positive keratinocytic area were significantly higher in group 1 than in group 2 cases. The subepithelial infiltrate in OLP cases showed weakly stained abundant Fas-positive mononuclear inflammatory cells either distributed evenly throughout the infiltrate or focally as clusters of positive cells. The subepithelial Fas-positive C.T. cells in both groups have a similar distribution but being more intensely stained in group 1 than in group 2. These data were in agreement with those of Neppelberg *et al.*, 2001 and Santoro *et al.*, 2004. The formers found the predominance of CD_3^+ and CD_4^+ lymphocytes in the OLP subepithelial infiltrate and also reported that CD_8^+ (cytotoxic) T-cells accumulation is associated with the disease progression and seemed to be more concentrated in the region subjacent to the basement membrane while CD_4^+ cells were more numerous in the deeper region of the subepithelial infiltrate. In addition, they observed a low rate of apoptosis in the OLP subepithelial T-cell infiltrate despite the prominent Fas/Fas L expression throughout this infiltrate. In contrary, Dekker *et al.* (1997) displayed indefinitive difference between Fas-mediated keratinocytes death in OLP and in normal oral mucosa.

Sklavounou *et al.* (2004) noticed that Fas-mediated apoptosis plays a vital role in the immune system and found significantly increased serum Fas (soluble splice variant of Fas) levels in OLP patients compared to those in healthy individuals. However, an association of serum sFas levels with the clinical form of OLP was not detected. Thus, a putative dysfunction in the Fas, Fas L mediated apoptosis might be involved in the pathogenesis of OLP as the sFas release may occur in T-cells escape from apoptosis leading to their accumulation locally in OLP. Moreover, Sugermann *et al.* (2000) identified anti-keratinocyte autocytotoxic T-cell clones in OLP suggesting a role for autoimmunity of that disease. Elimination of the putative autoreactive lymphocytes through Fas/Fas L mediated apoptosis may be bocked by an upregulation of sFas that may antagonize the Fas-membrane-bound form in OLP lesions. This hypothesis can explain the previously mentioned Neppelberg's *et al.* (2001) observation that a low rate of apoptosis in the subepithelial T-cell infiltrate was found despite the prominent Fas/Fas L expression throughout the OLP inflammatory cells.

Expression of P53 and Fas in our all cases (100%) may indicate the high sensitivity of the technique employed and the ideal fixed tissue used.

Finally, the superficial keratinocytes in our study didn't immunostain with Bcl-2, P53 or Fas molecule. This finding simulated what was revealed by Dekker *et al.*, 1997; *Majorana et al.*, 1999; Bloor *et al.*, 1999; Neppelberg *et al.*, 2001 & Khan *et al.*, 2003 who resorted that to the localization of the intraepithelial apoptotic cells to the site nearby the subepithelial inflammatory infiltrate of OLP which is involved in the process of apoptosis. This site will be the basal and suprabasal layers. Another possible cause that either of these molecules may be tied up by other proteins of Bcl-2 family.

We can conclude that Bcl-2, P53 and Fas proteins are operative in oral lichen planus. These proteins may be involved in persistence of the OLP lymphoid infiltrate and possibly, in keratinocyte apoptosis. Moreover, the P53 expression in OLP strongly suggests upregulation of its wild-type for the purposes of DNA repair or initiation of apoptosis.

Our study can give better understanding of the apoptosis process in OLP and hopefully can result in a future therapy of this disease.

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التساقط الخلوي المبرمج في مرض الحزاز الفمي

إظهار مناعي هستوكيميائي لجزئيات بي سي إل-2 ،بي 53، فاس :

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من أقسام: الباثولوجي[1،2] – والجلدية والتناسلية [4،3،5] ، بطب الأزهر أفرع أسيوط وبنين وبنات القاهرة، ومن قسم جراحة الفم واللثة والتشخيص بالأشعة[6] بطب أسنان بنات الأزهر بالقاهرة

التساقط الخلوي المبرمج قد يكون من الأسباب المساعدة لهدم خلايا الكيراتين القاعدية والجارقاعدية في مرض الحزاز الفمي، وكان هدف هذه الدراسة هو تقييم البروتينات المنظمة لهذه العملية، وقد أجريت هذه الدراسة على عشر حالات من مرض الحزاز الفمي كمجموعة أولى علاوة على عشر حالات من الغشاء المبطن للفم لأشخاص أصحاء كمجموعة ثانية ضابطة.

أظهرت الدراسة وجود جزئ بي سي إل-2 في عدد قليل من خلايا الكيراتين القاعدي وفوق القاعدي في نسبة 30٪ من حالات المجموعة الأولى، وفي عدد أكثر قليلا من هذه الخلايا في نسبة 20٪ من حالات المجموعة الثانية، ولم توجد أهمية إحصائية في متوسط النسبة المئوية لمناطق خلايا الكيراتين الإيجابية لهذا الجزئ بين المجموعتين. وكان هذا الجزئ عميقا في لون الصبغة في خلايا الميلانين والخلايا الليمفاوية وخلايا النسيج الضام لكل حالات المجموعة الأولى وكان أقل عمقا في خلايا الميلانين في كل حالات المجموعة الثانية. وبالعكس ظهرت خلايا الكيراتين الإيجابية لجزئ بي 53 في كل حالات المجموعتين (100٪) وبعدد أكثر في الخلايا القاعدية عنه في تلك الفوق قاعدية، وكان متوسط النسبة المئوية لمناطق خلايا الكيراتين الإيجابية لهذا الجزئ أعلى وذات دلالة إحصائية هامة (قيمة ب الإحصائية أقل من 0.0001) في المجموعة الأولى عنه في الثانية. وأظهرت الخلايا الليمفاوية في الأولى هذا الجزئ بدرجة لون متوسطة إلى عميقة.

وبالنسبة لجزئ فاس (Fas) فقد كانت أيضا جميع حالات المجموعتين إيجابية وعدد خلايا الكيراتين الإيجابية في المجموعة الأولى أكثر كثيرا منه في المجموعة الثانية، وكان متوسط النسبة المئوية لمناطق خلايا الكيراتين الإيجابية لهذا الجزئ أعلى وذات دلالة إحصائية هامة (قيمة ب الإحصائية أقل من 0.0001) في المجموعة الأولى عنه في الثانية.

كذلك أظهرت كثير من الخلايا الليمفاوية هذا الجُزئ ووزعت هذه الخلايا الإيجابية بالتساوي خلال النسيج الليمفاوي أو بؤريا على هيئة تجمعات في هذا النسيج لحالات المجموعة الأولى التي تشابه فيها توزيع خلايا النسيج الضام الإيجابية مع توزيعها في المجموعة الثانية وإن كانت أعمق في درجة الصبغة لهذا الجزئ في الأولى عنه في الثانية، ولم تظهر الطبقة السطحية الكيراتينيه أي من هذه الجزيئات.

وقد أثبتت هذه الدراسة الدور العملي لهذه الجزيئات في مرض الحزاز الفمي وقد تشترك في المحافظة على النسيج الليمفاوي المصاحب لهذا المرض وأيضا في التساقط الخلوي المبرمج لخلايا الكيراتين كما تمدنا هذه الدراسة بمفهوم أحسن لعملية التساقط الخلوي المبرمج والذي نأمل أن يساعد في العلاج المستقبلي لهذا المرض.