

# Immunohistochemical Analysis of PCNA and P53 Proteins in Oral Lichen Planus, Oral Dysplasia and Normal Oral Mucosa

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## Abstract

**Background:** The overexpression of the protein products of genes associated with the cell cycle protein p53 and PCNA is apparently of great significance. Oral lichen planus classified among the potentially malignant lesions of oral mucosa. The aim of this study is to evaluate and compare the tissue expression PCNA and p53 proteins in oral lichen planus, epithelial dysplasia cases and normal oral mucosa.

**Materials and methods:** Formalin-fixed and paraffin- embedded blocks of 21 lichen planus, 21 oral dysplasia and 10 of normal oral mucosa cases were used in immunohistochemical (IHC) analysis for PCNA and p53 monoclonal antibodies.

**Results:** The mean of PCNA expression in OLP (44.14) was less than in the control group (74) and there was an opposite result of p53 (12.19) when compared with the control group (1.05), and in cases of epithelial dysplasia the same result was found and the mean PCNA value in epithelial dysplasia was significantly higher than those found in controls and in oral lichen planus. There was a statistically significant difference between the expression of p53 and PCNA proteins in oral lichen planus cases and non- significant differences of both proteins expression in oral dysplasia cases. Moreover, there was a significant positive correlation between the numbers PCNA and p53 percentage of positive cells in oral lichen planus group.

**Conclusion:** The proportion of cases with positive PCNA and p53 expressions increased from normal oral mucosa to lichen planus to epithelial dysplasia. These results may indicate the presence of high degree of PCNA and p53 proteins in chronic and premalignant lesion like oral lichen planus and epithelial dysplasia can be great helpful in their prognosis and suggested treatment.

**Key words:** oral lichen planus, epithelial dysplasia, PCNA, p53.

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## Introduction

Oral lichen planus (OLP) is a common chronic inflammatory and dermatological disorder of the oral mucosa and skin [1, 2]. This inflammatory disease is generally regarded as a benign pathology [3]; however, transformation towards malignant condition in some cases has forced many physicians to

consider OLP as a premalignant entity [4]. WHO classifies OLP as a premalignant condition; however, the underlying mechanisms initiating development of cancer in OLP lesions are not understood [5]. Although the presence of dysplasia does not always indicate malignant transformation and its absence does not preclude it [6].

Moreover, histological assessment of dysplasia is extremely subjective and prone to inter and intra – observer variation [7], and additionally some potentially malignant lesions do not show dysplastic alterations [8, 9, 10].

Oral lichen planus lesions are characterised by the epithelial cells of the basal layer undergo a distinct process of cellular death, which is called apoptosis [11] and histopathologically is characterized by hyperkeratosis, degeneration of the basal cell layer and a dense lymphocytic infiltration in a band like pattern in the lamina propria [12, 13].

The detection of antigens related to cell cycle by immunohistochemical methods can contribute for an understanding of biological dynamics to pathological condition and the important markers are PCNA and p53.

The PCNA (proliferating cell nuclear antigen) is an auxiliary enzyme of the DNA polymerase –delta that is involved in the DNA synthesis during the S – phase of the cell cycle, and its detection have been used to evaluate cell proliferation [14].

Previous reports demonstrated the application of monoclonal antibodies against PCNA, since this is a fine indicator of the biological behaviour of some premalignant and malignant lesions [15].

The normal p53 gene acts as a tumour suppressor and it is involved in cell cycle control, apoptosis, and the preservation of genomic stability [16]. Cells that contain p53 genes of the wild type are able to delay cell cycle to allow the repair of damaged DNA, or divert the cell into apoptosis. When the protein is mutated or absent, the cells replicate the damaged DNA, which will result in more mutations and chromosome rearrangement. The mutant form of p53 protein is stable, has an extended half life, and can be detected by immunohistochemistry [17, 18]. Aim of the study is to evaluate and compare the tissue

expressions of PCNA and p53 proteins in oral lichen planus, epithelial dysplasia and normal oral mucosa groups.

## Materials and Methods

Twenty-one cases of oral lichen planus and 21 cases of epithelial dysplasia (3 mild, 11 moderate, 7 severe) obtained from the archives of the department of Oral Diagnosis of Collage of Dentistry – University of Baghdad from the period between (2000–2012) were included in the study. The normal oral mucosa tissues (10 samples) were collected from patients attends to department of periododontology. Age and gender of patient was not considered in the study.

Three 5µm thick histological sections were cut from the formalin -fixed paraffin-embedded blocks .One section was stained with H&E to verify the histological diagnosis; the remaining two sections were employed for immunohistochemical analysis. The cases of oral lichen planus were selected according to Eisenberg’s criteria [19] and World Health Organization’s criteria for epithelial dysplasia [20]. Cases of oral lichen planus with doubt of epithelial dysplasia were excluded.

## Immunohistochemical (Ihc) Method

From each case 5 µm thick sections were cut and mounted on positively charged slides. Sections were deparaffinized and rehydrated. For antigen retrieval, the sections for PCNA and p53 antigen immunostaining were microwaved treated in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked for 10 minutes in H<sub>2</sub>O<sub>2</sub> and methanol. Subsequently, sections were washed with phosphate-buffered saline and incubated at 4°C with the PCNA monoclonal antibody PC10 (dilution 1:50, DAKO CO, Glostrup, Denmark) and p53 monoclonal antibody DO-7(dilution 1:80, DAKO CO, Glostrup, Denmark. After wash with phosphate-buffered saline, the following step was the incubation with the streptoavidin-biotin

complex (dilution 1:100) overnight at 37°C temperature. The reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution. The sections were counterstained with Mayer's haematoxylin for optimal evaluation and cell counting.

Paraffin – embedded oral squamous cell carcinoma biopsy cases served as positive control. As negative control primary antibodies were replaced and with antibody diluent solution.

### **The Evaluation and Scoring System**

After the immunohistochemical reactions, only cells that presented brown – coloured staining were considered positive and the intensity was excluded. PCNA and p53 expression was classified according to the number of positively stained epithelial cell per 1000 cells. A semiquantitative assessment was applied by counting cells randomly at the basal and suprabasal epithelium cell layers. The percentage of positive cells was scored according to the method of Cruz et al. <sup>(21)</sup> as follows: (+++) = strong staining (more than 50% stained); (++) = moderate staining (25 – 50% stained); (+) = weak staining (5 – 25%); 0 = negative (less than 5% stained).

### **Statistical Analysis**

The statistical analysis was performed by using a software package. Data were analysed by one way analysis (ANOVA) test. The mean ± SD were collected of each group by summation of percentage of positive cells.

The level of significance was considered at  $p \leq 0.05$ . Pearson's correlation test was used to verify any correlation between the percentage of positive cells of PCNA and p53 into oral lichen planus cases.

## **Results**

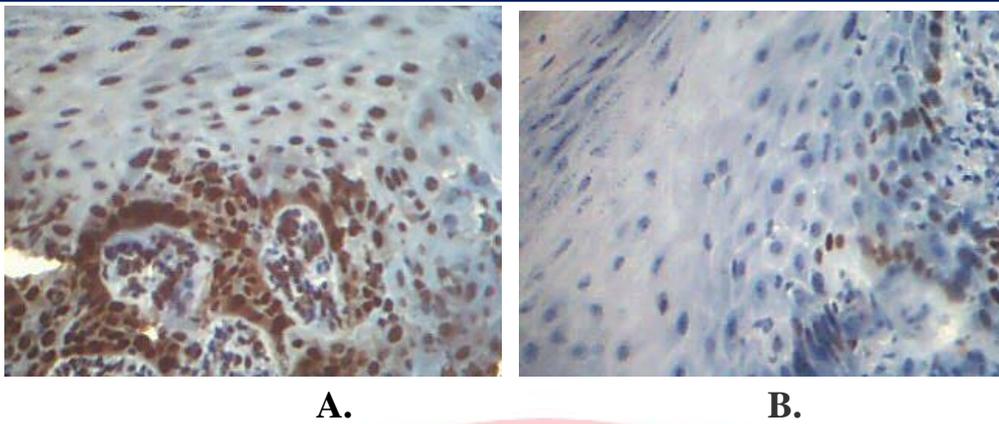
The PCNA and p53 positive cells showed immunoreactivity restricted to the cellular nuclei of epithelia as shown in figures 1 (A&B), 2(A&B) and 3(A&B). In the normal oral mucosa cases immunostaining for PCNA was limited exclusively to the lower third of the epithelium while p53 expression was observed in only a few cells of the basal layer while the spinous cell layers were devoid of p53 antigen expression.

The majority of positive immunostaining for PCNA was observed in 18/21(85.7%) of the oral lichen planus cases, 19/21(90.5%) of the epithelial dysplasia cases and positive nuclear staining of p53 was found in 11/ 21 (52.4%) cases of lichen planus and 17/21 (80.9%) cases of epithelial dysplasia.

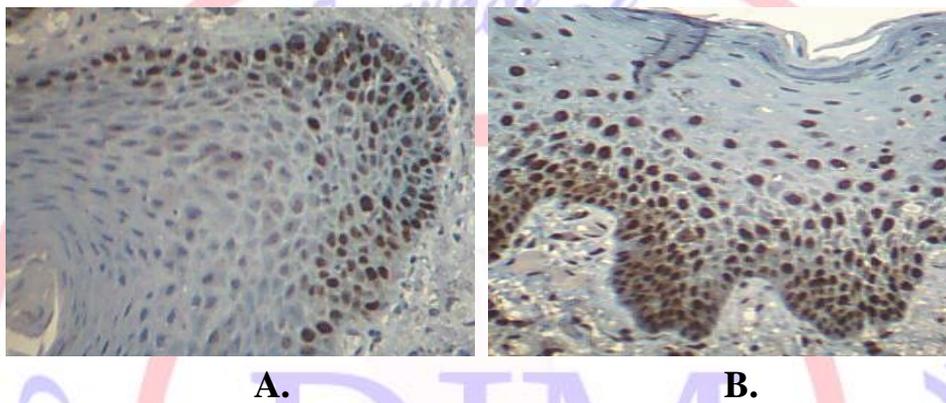
The mean of PCNA expression in OLP (44.14) was less than in the control group (74) and there was an opposite result of p53 (12.19) when compared with the control group (1.05), and in the cases of epithelial dysplasia the same result was found.

One-way ANOVA showed that the mean PCNA value in epithelial dysplasia was significantly higher ( $F=4.442$ ;  $p<.01$ ) than those found in controls and in oral lichen planus. These differences were statistically significant as shown in table 1.

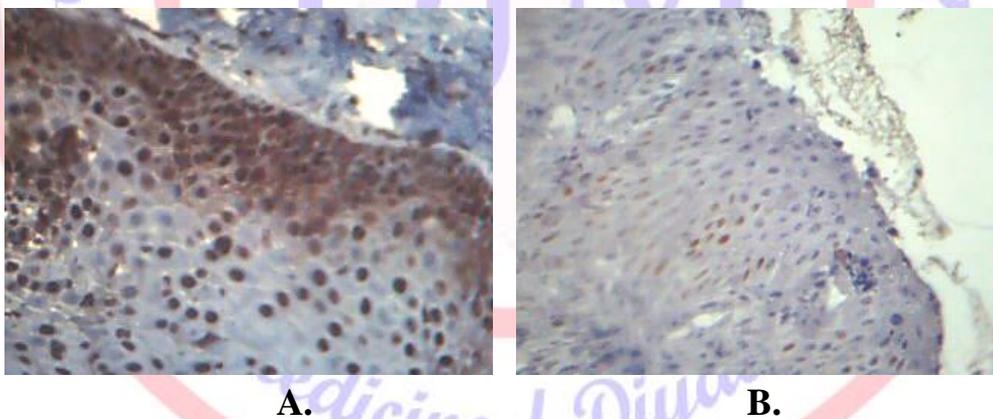
There was a statistically significant difference between the expression of PCNA and p53 proteins in oral lichen planus whereas there was no significant differences of both proteins expression in oral dysplasia. Moreover, Pearson's correlation test was also able to demonstrate significant positive correlation between the numbers PCNA and p53 of percentage of positive cells in oral lichen planus group as shown in figure 4.



**Figure (1):** oral lichen planus (A: PCNA X200; B: p53 X200).



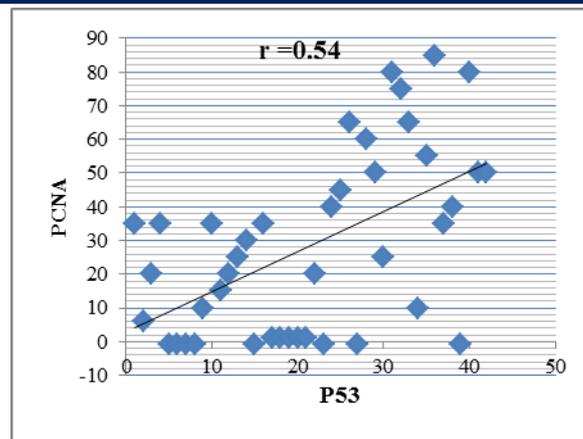
**Figure (2):** Oral dysplasia (A: PCNA X200; B: p53 X200).



**Figure (3):** normal oral mucosa (A: PCNA X200; B: p53 X200).

**Table (1):** Frequency of PCNA and p53 protein expression in oral lichen planus, Oral dysplasia and normal oral mucosa.

Variable	Group	No.	Mean± SD	P-value
PCNA	NOM	10	74±13.904	F-test=4.442 P=0.017 S
	OLP	21	44.14±27.27	
	E Dys.	21	52.04±29.05	
p53	NOM	10	1.05±1.623	F-test=20.55 P<0.01 HS
	OLP	21	12.19±14.95	
	E Dys.	21	51.71±34.81	



**Figure (4):** Correlation between PCNA and p53 in oral lichen planus lesion.

## Discussion

The assessment of changes at the molecular level may become the primary means of diagnosis and may guide management since these changes mediate morphologic changes that occur after genetic changes, and knowledge of current morphologic changes is based on the subjective assessment of clinical and histopathologic changes [10, 18].

p53, a tumour suppressor protein, acts as a "molecular brake" to critically regulate the cell cycle. This DNA-binding protein has also been involved in DNA repair and synthesis, cell proliferation, cell differentiation, programmed cell death, and in the maintenance of genomic stability. As p53 protein has been reported to be expressed at high levels in malignant lesions [6, 18] assessment of its levels in premalignant lesions is significant.

In the result of this study the cases of oral lichen planus with elevated percentage of positive cells for PCNA can have a higher malignant transformation risk. In addition, the presence of the positive cells for PCNA in superficial layer of epithelium suggests possible alterations in cell differentiation mechanisms, step essential to the malignant transformation of epithelium and this result was in agreement other reports [22 – 25], for those authors that indicate, the alterations in the expression of these proteins were a strong

indicator of the potential for malignant transformation of oral lichen planus, as these proteins participate actively in oral carcinogenesis.

PCNA has been used as a marker of cell proliferation, but some reports attempt that its presence has been shown to represent both induction by growth factors and excision repair processes [14]. It is known that the inflammatory cells are a potential source of some growth factors [15, 22, 23, 25, 26]. In OLP lesions exist a characteristic chronic inflammatory infiltrate, and it could be expected a high of PCNA expression in OLP. Moreover, it was observed in the present study a significant difference between OLP and epithelial dysplasia. Thus, these results suggest that the similar PCNA expression in the two examined groups (OLP & ED) indicate the real proliferative potential of the epithelia and described by other authors [22, 23]. In addition, other studies using ki-67 in OLP identified compatible results with the ones of this study [4, 24].

In the group of normal oral mucosa all specimens showed weak p53 immunoexpression as reported by other investigators [24, 26]. Immunohistochemical detection of p53 protein does not necessarily indicate mutations p53 gene and malignant transformation, therefore, detection of p53 has been documented in a number of benign conditions, supporting that wild-type protein

can be observed in some circumstances where cell damage has occurred. Moreover, the DO-7 clone reacts with both wild-type and mutants forms of p53, and so there is no exact correspondence between p53 positivity and presence of p53 mutation [27].

The mechanism underlying p53 over-expression in OLP is also subject to controversy. The expressions of p53 and active proliferation status in OLP have also been reported by a few authors [25, 26]. A number of authors estimate that p53 over-expression constitutes a form of cell response to the hyper-proliferative state frequently seen in OLP [17, 25 – 27].

### Conclusion

The result of the study may indicate the presence of high degree of PCNA and p53 proteins in chronic and premalignant lesion like oral lichen planus and epithelial dysplasia can be great helpful in their prognosis and suggested treatment and it must be emphasised that at least some OLP lesions could have an intrinsic property predisposing neoplastic transformation, even in small percentage. Therefore the most appropriate management on OLP patients needs a careful evaluation.

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