

Immunohistochemical expression of p53 and PCNA proteins in oral lichen planus and oral dysplasia

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ABSTRACT

Background: Oral lichen planus (OLP) is a relatively common chronic inflammatory muco-cutaneous disease classified among the potentially malignant lesions of oral mucosa. The aim of this study is to investigate and compare the expression of p53 and PCNA proteins in oral lichen planus and epithelial dysplasia cases.

Materials and methods: Formalin-fixed and paraffin- embedded blocks of 21 lichen planus and 21 oral dysplasia cases were referred to immunohistochemical (IHC) analysis for anti p53 and anti PCNA monoclonal antibodies.

Results: The results showed that positive nuclear staining for p53 was found in 11/21 (52.4%) cases of lichen planus and 17/21 (80.9%) cases of dysplasia. Positivity for PCNA was observed in 18/21 (85.7%) of oral lichen planus cases, and 19/21 (90.5%) of epithelial dysplasia cases. There was a statistically significant difference between the expression of p53 and PCNA proteins in oral lichen planus cases and non- significant differences of either protein expression in oral dysplasia cases. No statistically significant difference of p53 and PCNA proteins expression between oral lichen planus and epithelial dysplasia cases was found. Moreover, there was no significant difference in P53 and PCNA expression with respect to the grade of epithelial dysplasia.

Conclusion: The proportion of cases with positive p53 expression increased from lichen planus to dysplasia. These results may indicate an involvement of p53 in neoplastic transformation as well as in proliferative events PCNA, although the absence of p53 staining could be used to predict the outcome of potentially malignant oral mucosal lesions.

Key words: Oral lichen planus, oral dysplasia, p53, PCNA. (J Bagh Coll Dentistry 2014; 26(1):98-102).

INTRODUCTION

Lichen planus is a chronic inflammatory disease of the oral cavity in 1-5% of general population⁽¹⁾ the disease represents as a set of lesions including white involvements (striation, papule, plaque), erythema, erosions and blisters mainly on the mucosa, gingival structures and tongue⁽²⁾. Oral lichen planus is not a rare condition in human being. This inflammatory disease is generally regarded as a benign pathology; however, transformation towards malignant condition in some cases has forced many physicians to consider L.P as a premalignant entity⁽³⁾. Since the first case was reported in 1910, several studies have suggested that patients with oral lichen planus are at an increased risk of developing cancer^(4,5).

However; many authors believe that there is insufficient data to prove an association between oral lichen planus and cancer. For these authors, most cases of malignant transformation are the result of errors in the initial diagnosis of the disease^(4,6). Since the first case reported of squamous cell carcinoma developing from a mucosal LP, the real odds of such transformation, is a matter of discussion.

Thus, several studies have shown different proportions of malignant potential of OLP, many authors have accepted the idea that OLP is an actual premalignant lesion^(7,8). The true potential for malignant transformation of oral lichen planus can be evaluated by analyzing the expression of proteins related to cell proliferation and apoptosis, as alternations in the expression of these proteins are essential for carcinogenesis^(9,10).

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 mutation and chromosome rearrangement. Mutations in the P53 tumour suppressor gene are the most common molecular defects in human malignancies including oral squamous cell carcinoma⁽⁷⁾.

The normal p53 gene acts as a tumour suppressor and its wild type acts inhibiting proliferation and oncogene – mediated proliferation and transformation. Cells that contain P53 genes of the wild type are able PCNA (proliferating cell nuclear antigen) is a nuclear acid protein of 36 Kda, which works as an auxiliary protein of delta polymerase, associated to the DNA duplication and repair⁽¹¹⁾. The concentration of PCNA is variable during the steps of cell cycle, being higher in late G1 phase, with peaks in G1/S phase, being practically absent in G2 and M phases⁽¹²⁾.

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Several studies described the application of monoclonal antibodies against PCNA, since this is a fine indicator of the biological behaviour of some premalignant and malignant lesions⁽¹³⁾. Aim of the study is to investigate and compare the expression of PCNA and p53 proteins in oral lichen planus and epithelial dysplasia cases.

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 files of the department of Oral Diagnosis of College of Dentistry – University of Baghdad from the period between (2000– 2012) were included in the study. Age and gender of patient were not considered because these data are not related to the increase of the risk of malignant transformation of oral lichen planus. 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⁽¹⁴⁾ and World Health Organization's criteria for epithelial dysplasia⁽¹⁵⁾. Cases of oral lichen planus with doubt of epithelial dysplasia were excluded.

Immunohistochemical 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 H₂O₂ 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 biopsied cases served as positive control. As negative control primary antibodies were replaced and with antibody diluent solution.

Scoring system

After the immunohistochemical reactions, PCNA and p53 expression was classified according to the number of positively stained epithelial cell per 1000 cells. A quantitative assessment by 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 Nakagawa et al.⁽¹⁶⁾ 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

Data were analysed by Chi – square test. The expressions of both p53 and PCNA in epithelial dysplasia according to their grading were analyzed statistically by (ANOVA) test. Value of $P \leq 0.05$ was considered statistically significant.

RESULTS

It was considered a positive cell if it exhibited a reaction represented by a brownish staining independent of the intensity. The p53 and PCNA positive cells showed immunoreactivity restricted to the cellular nuclei of epithelia as shown in figures 1(A&B) and 2(A&B). The expression of PCNA, in oral lichen planus, was observed in the basal and lower suprabasal layers. While its expression, in dysplastic epithelium, was observed in the suprabasal and lower spinous layers; and the intensity of staining increased along the degree of cellular atypia. The expression of p53, in oral lichen planus, was localized in the basal and lower suprabasal layers and in dysplastic cases was limited to the basal and suprabasal layers. In one case of dysplasia, weak positive immunoreactivity for p53 could be seen in a few isolated cells of the basal layer. Positive nuclear staining of p53 was found in 11/ 21 (52.4%) cases of lichen planus and 17/21 (80.9%) cases of dysplasia, while the positivity for PCNA was observed in 18/21(85.7%) of the oral lichen planus cases, 19/21(90.5%) of the epithelial dysplasia cases as shown in figures 3 and 4 respectively. Chi-squared test showed that the number of positive cases for p53 and PCNA was significantly lower in oral lichen planus than in oral epithelial dysplasia ($p < 0.05$). There was a statistically significant difference between the expression of p53 and PCNA proteins in oral lichen plauns whereas there was no significant differences of both proteins expression in oral dysplasia .No statistically significant difference of p53 and PCNA proteins expression between oral lichen planus and epithelial dysplasia was found ($p > 0.05$). There was no significant difference

regarding p53 and PCNA expression in relation to the grade of epithelial dysplasia as demonstrated in figure 5.

DISCUSSION

It is almost a long time that there is an ongoing debate on the nature of oral lichen planus. Despite a number of studies in the literature in this regard, there is not yet a consensus^(2-4,6). This is because the higher the cell proliferation rate, the higher the risk of cells suffering mutations during mitosis, which could result in malignant phenotype. P53 tumour suppressor gene which is a frequent target for mutations in a high percentage of oral cancer is regarded as an early event in carcinogenesis^(3,6,9,17,18).

In context, the cases of oral lichen planus with elevated percentage of positive cells for PCNA (more than 50% of cell stained) can have a higher malignant transformation risk. In addition, in these cases, 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.

To depict this heterogeneity Van der Meijetal⁽¹⁹⁾ found no potential risk of transformation toward SCC in their cases with oral lichen planus, while Acayetal⁽⁷⁾ concluded that oral lichen planus could be regarded as a premalignant condition. According to Stoll et al., the loss of p53 function is found in at least half of oral cancer cases. Therefore, the similar expression of p53 in oral lichen planus and in epithelial dysplasia can be an important indicator of malignant transformation potential of these lesions⁽²⁰⁾.

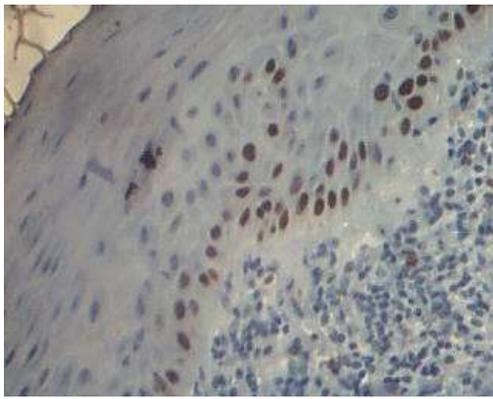
Immunohistochemical detection of p53 protein does not necessarily indicate p53 gene mutations and malignant transformation, therefore, detection of p53 has been documented in a number of benign conditions, supporting that wild-type protein can be observed in some circumstances where cell damage has occurred. 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 the presence of p53 mutation^(21,22). Alteration in the expression of the proteins related to cell proliferation and apoptosis is a strong indicator of the malignant transformation potential of a certain lesion⁽²³⁾. The obtained results suggested that oral lichen planus presents a possibilities of evolution to cancer similar to epithelial dysplasia. Therefore, cases of malignant transformation of oral lichen planus are not just consequences of error in their initial diagnosis, but natural evolution of this disease.

In the present study, we examined the rate of p53 and PCNA expressions in cases with oral lichen planus and epithelial dysplasia. Based on the immunohistochemical evaluation, p53 was significantly more prevalent in cases with epithelial dysplasia than in oral lichen planus cases. These findings indicate that there might be a potential tendency for malignancy in oral lichen planus. This result is in agreement with those of several other authors who evaluated the expression of p53 and PCNA in addition to other proteins related to cell proliferation and apoptosis in oral lichen planus^(24,25). For these authors, the alteration in 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.

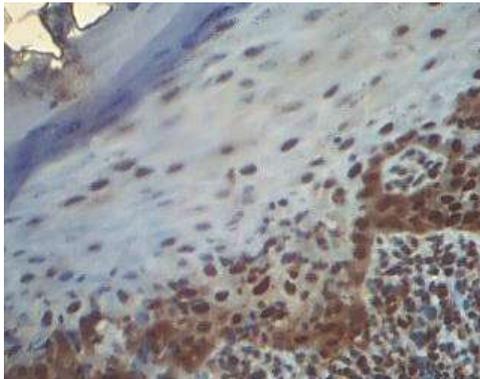
The results obtained from the present study showed that the number of positive PCNA cases in oral lichen planus is lower than in epithelial dysplasia, although it had no significant statistical difference between these two lesions. This fact suggests that, in general, the cell proliferation rate in oral lichen planus is lower than in epithelial dysplasia. Therefore, oral lichen planus has a lower possibility to accumulate genetic mutation than the epithelial dysplasia and, consequently, a lower possibility to suffer malignant transformation. This is because the higher the cell proliferation rate, the higher the risk of cells suffering mutation during mitosis, which could result in malignant phenotype. According to Lee et al⁽²⁶⁾, the expression of PCNA in oral lichen planus is similar to hyperkeratosis, but superior to normal mucosa and inferior to epithelial dysplasia and oral squamous cell carcinoma, being in accordance to the results obtained in these studies. However, for Da Silva Fonseca and Do Carmo⁽²⁷⁾, the higher cell proliferation rate in oral lichen planus than in hyperkeratosis and normal mucosa makes it more susceptible to the action of carcinogens. In conclusion, the proportion of cases with positive p53 expression increased from lichen planus to dysplasia. These results may indicate an involvement of p53 in neoplastic transformation as well as in proliferative events PCNA, although the absence of p53 staining could be used to predict the outcome of potentially malignant oral mucosal lesions. Therefore, there is a need for long-term rigorous follow-up of patients with oral lichen planus, aiming at precocious identification of an alteration that can indicate a possible malignant transformation.

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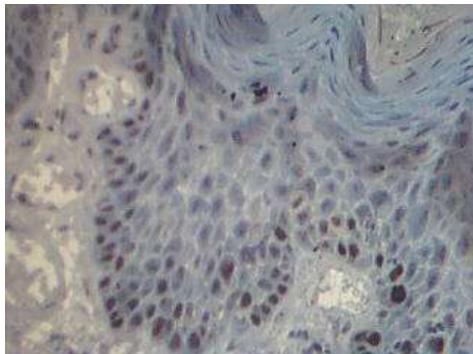


A.

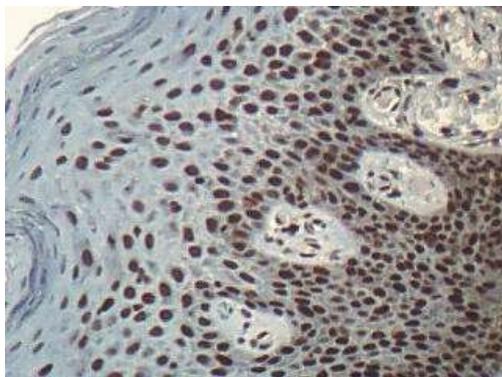


B.

Figure 1: Oral lichen planus (A: P53 X200; B: PCNA X200).



A.



B.

Figure 2: Oral dysplasia (A: P53 X200; B: PCNA X200).

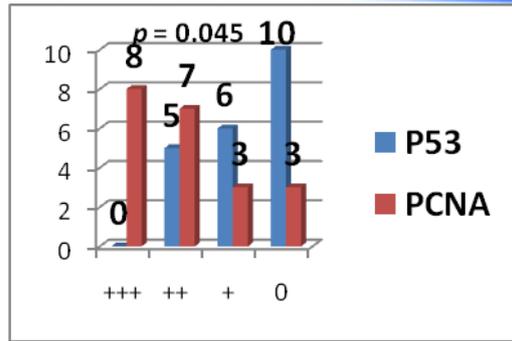


Figure 3: Expression of p53 and PCNA in oral lichen planus.

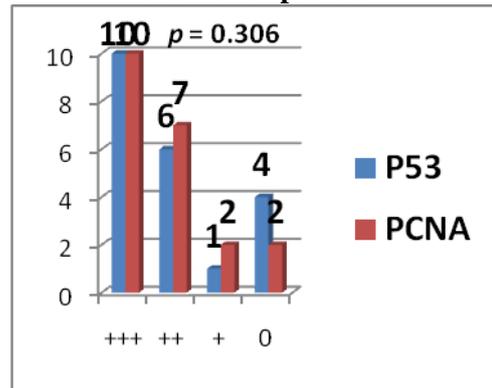


Figure 4: Expression of p53 and PCNA in oral dysplasia.

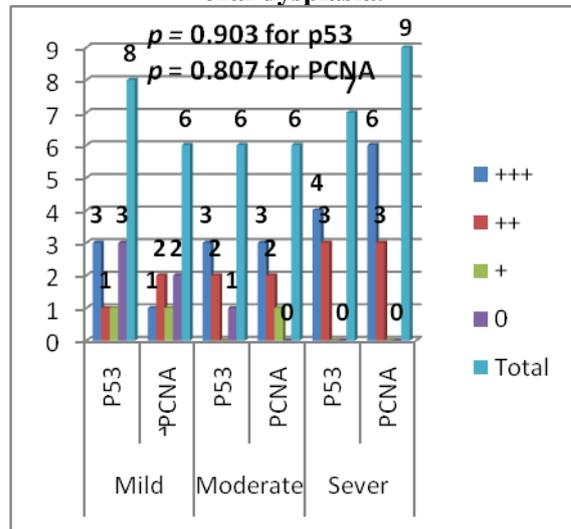


Figure 5: Expression of p53 and PCNA in relation to the grading of oral dysplasia.