Evaluation of syndecan-1 and p53 protein in patients with oral lichen panus

Asmaa Ali Hussein
College of Medicine, University of Fallujah, IRAQ
mmss2006@uofallujah.edu.iq

Abstract
Oral lichen planus disease is considered as inflammatory chronic mucocutaneous unknown etiology with a potential for malignant transformation. Little is known about the syndecan-1 and p53 protein expression of in Iraqi patients with Oral lichen planus Disease. The current study aimed to evaluate syndecan-1 and p53 protein expression in Iraqi section of Oral lichen planus using immunohistochemical technique. Sections of 30 formalin-fixed paraffin embedded blocks specimens of Oral lichen planus were immunostained to assess the expression of syndecan-1 and p53.

The syndecan-1 and p53 expression were positive in all oral lichen planus cases (100%). The positive expression of p53 was significantly correlated with the site (p=0.016) and clinical presentation (p-value=0.003). The positive syndecan-1expression was significantly statically correlated with tumor site (p-value =0.002). On other hand there was non-significant correlation between p53 and syndecan-1 (p-value= 0.021). The syndecan-1 and p53 positive expression was noted in all cases of oral lichen planus cases signifying their important role in the inflammation, suggesting that can be used for the development of anti-inflammation therapeutics as targets for head and neck malignancies.

Keywords: Syndecan-1, p53, immunohistochemical technique.

Introduction

Immunohistochemistry (IHC) technique is a method for detecting the presence and location of syndecan-1 and p53proteins in tissue sections[1]. Although quantitatively less sensitive when compared with immunoassays like ELISA and western blotting, it enables in the context of intact tissue the processes observation[2]. This is useful for treatment and assessing the progression of diseases such as cancer. In general, the information cleared from IHC with microscopy literally provides a "big picture" that leads to make sense of data obtained using molecular analysis. Immunostaining in combined antibodies helps in recognize the protein target[3].

The syndecans are a protein family of four transmembrane heparan sulfate proteoglycans associated with the surface cell, matrix extracellular and consist of a core protein covalently attached[4]. The syndecan protein consists of four members[5]. It participates in cell proliferation, cell migration and matrix interactions by its receptor for extracellular matrix proteins which can bind to interstitial matrix including fibronectin, both cell-cell and cell-extracellular matrix interactions[6].

Overexpression of p53 by immunohistochemistry (IHC) has been identified in 11–55% of oral lichen plauns (OLP) in the world[7]. Expression of *p53* is induced by diverse forms of cellular stresses such as hypoxia or DNA damage caused mediating cell response to various stresses, mainly by inducing or repressing a number of genes involved in cell cycle arrest, senscence, apoptosis, DNA repair and angiogenesis[8].

Material and Methods

Sample collection: The sample of this study included thirty formalin-fixed, paraffin- embedded tissue blocks which have been diagnosed as oral lichen planus dated from January 20017 till February 2008. The study samples were obtained from the Department of Oral and Maxillofacial Pathology/ College of Dentistry/ University of Baghdad (20) blocks; and private laboratories in Baghdad (10) blocks. The diagnosis of each case was confirmed by examining the Hematoxylin and Eosin (H and E) sections by two specialized pathologists. Demographic and clinical data provided by the surgeon were obtained from the surgical and pathological reports available with the tissue specimens including patient's age, sex, clinical presentation.

The positive control was obtained according to antibodies manufacturer's data sheet. Slides were prepared from blocks of patient having tissue known to contain the target antigen[9]. Negative control was used for indicating the properness of the staining techniques as positive control; One negative control was used during the experiments run. All reagents except the primary antibody were applied. Positive staining indicates a lack of specificity of the antibody.[10]

Tissue preparation and staining:

A. Tissue specimens - All samples and controls were presumably fixed in 10% formalin and processed routinely into paraffin blocks.

B. Sections - From each paraffin embedded tissue block (samples and controls); serial sections were cut as follows:

- Sections of 4µm thickness were mounted on normal glass slides, stained with H and E and re-evaluated histopathologically. Histological grades were recognized for each case by two specialized pathologists.

- Two other 4µm thick sections for each case were cut and mounted on positively charged slides (Fisher scientific and Escho superfrost plus, USA for immunohistochemical staining with monoclonal antibodies[11,12].

Immunohistochemical detection Kit of syndecan-1 and p53 Antibodies: For immunohistochemical (IHC) evaluations, the sections were deparaffinized in the hot air incubator at 80ºC for 70 min using adhesion microscope positively charged slides and then rehydrated in graded alcohols[13]. Backed slides were immersed sequentially at room temperature for the indicated times in the following solutions: Xylene for 30 minutes, Fresh xylene for 30 minutes, absolute ethanol for 5 minutes, 90% ethanol for 5 minutes, 70% ethanol for 5 minutes, 50% ethanol for 5 minutes and distilled water for 5 minutes. These sections were subjected to IHC evaluations using polyclonal anti-syndecan-1 and p53 antibody[14]. The slides were cooled for 20 minutes at room temperature and then the edges surrounding the sections were marked by a liquid blocker pap pen to avoid the distribution of the materials out of the sections during the run of the IHC staining[15].

Then, the slides were transferred quickly to the strainer racks to avoid drying of samples. Enough drops of hydrogen peroxide were added and blocked to cover the sections and then incubated for 10 minutes and washed two times in the buffer, then 20µL of Protein block was applied and incubated for 10 minutes at room temperature to block nonspecific background staining and then washed one time in the buffer[14]. 40µl of primary antibody (anti- syndecan-1 and p53) was placed onto the section tissue and then incubated for 30 minutes at 37˚C in a chamber with humid.

The slides were drained and blotted gently and then transferred to the refrigerator for 24 hrs. After 24 hrs, the slides were placed in buffer bath for washing for 5 min, drained and blotted gently and 20µl of the secondary antibody (the complement) was applied onto the sections and the slides were placed in a humid chamber and incubated at 37ºC for 10 min, rinsed and placed in washing buffer bath as before, excess buffer drained and blotted gently. After that, 20µl of HRP conjugate was placed onto each tissue section and incubated for 15min at 37˚C in a humid chamber; the slides were placed in washing buffer bath for 5min, drained and blotted gently[16].

DAB Chromogen was added to DAB Substrate (one drop to 50 drops), then mixed by swirling and then applied to the tissue and incubated for 1-10 min, then rinsed 4 times in buffer. The slides were immersed in a bath of Mayer's Hematoxylin for 1 min and washed three times in distilled water 1 min each; then drained and blotted gently and dehydrated by placing the prepared slides in the following solutions: 50% ethanol for 5 min, 70% ethanol for 5 min, 90% ethanol for 5 min, absolute ethanol for 5 min, xylene for 5 min and fresh Xylene for 5 min. Finally, a drop of DPX was applied to the xylene wet sections and covered with cover slips gently to remove excess and air bubbles and then left to dry overnight.[17]

Evaluation of Immunostaining for p53 and syndecan-1 protein Expression: The expression of p53 protein was measured by counting the number of positive cells with brown (DAB) cytoplasmic staining under light microscopy 40X. For the evaluation of p53 expression, immunostaining was assessed semi quantitatively using a scoring system syndecan-1protein quantified by counting at least one thousand cells in representative five fields at 40X objective in each case. The extent of staining was scored using the following scale: 0 = no staining (negative), 1 =staining of 1–25% of cells (weak positive), 2 = staining of 26–75% of tumor cells (moderate positive), 3 =staining of 76– 100% of tumor cells (strong positive)[18] According to protocol, cut off values of Scoring of p53 was used according to Gupta et al[19].

Positive nuclei staining of p53 in at least 10 % of the cell nuclei were considered p53 overexpression, while those with less than 10 % positive cell nuclei were considered normal expression. (Negative, score 0; weak or mild staining (5 to < 10 % score 1); moderate staining (10 to < 25 % score 2); strong staining (25 to < 50 % score 3) and highly strong staining (over 50 % score 4). Positive nuclei staining of p53 in at least 10 % of the cell nuclei were considered p53 overexpression, while those with less than 10 % positive cell nuclei were considered normal expression. (Negative, score 0; weak or mild staining (5 to < 10 % score 1); moderate staining (10 to < 25 % score 2); strong staining (25 to < 50 % score 3) and highly strong staining (over 50 % score 4).[20]

Statistical Analyses: Chi-square test and mean ± S.D. were used for the clinicopathological studies. All the statistical analyses were carried out in SPSS version 13.0 (SPSS, Inc., Chicago, USA) and Microsoft Excel.[21]

Results and Discussion

The highly specific of antibodies makes it bind only to the interest protein in the section of tissue[22]. The interaction between antibody-antigen in current study was visualized using chromogenic detection and or fluorescent detection. Protocol of IHC-Paraffin (IHC-P) refers to the tissues staining formalin fixed and then before being sectioned embedded in paraffin[23].

Evaluation of syndecan-1 protein Immunohistochemistry: Positive syndecan-1 protein Immuno staining was found in all oral lichen plauns cases as brown membranous or membranous and cytoplasmic expression as in figure 1. Syndecan-1 protein immuno staining of the oral lichen plauns cases is summarized in table 1 which reveals that (4) cases (13.3%) showed weak positive expression, (11) case (36.7%) showed moderate positive expression and (15) cases (50.0%) showed strong positive expression.

Evaluation of p53 Immunohistochemistry: Positive p53 immunostaining was detected as brown nucleus staining of the tissue cells as in figure 2. Positive IHC expression was found in all oral lichen plauns cases as illustrated in table 2 which reveals that (3) cases (10.0%) showed weak positive expression, (9) cases (30.0%) showed moderate positive expression and (18) cases (60.0%) showed strong positive expression.

Statistical correlations of all studied immunohistochemistry IHC markers: Pearson's correlation between two variables is defined as the covariance of the two variables divided by the product of their standard deviations.

The mode of correlations between the P53 and syndecan-1 markers in the oral lichen plauns (OLP) studied statically according to the Pearson correlation as shown in the table 3, the P53 and syndecan-1 correlation manner was significant between markers with probability (p 0.001) as in table 3.

The present finding was in agreement with previous reports.[22-25] This suggests that syndecan-1 protein may be involved in mitoses seen in squamous cells of oral squamous cell carcinoma[26]. It has been demonstrated that syndecan-1 protein promotes the production of cancer cell proteinases and enhances their invasive ability. Is to be expected that syndecan-1 protein produced by cancer cells activates the cancer cells themselves and/or the fibroblasts for the invasion and growth of the cancer[27]. Many evidences demonstrated that syndecan-1 protein pathway contributes to the redundancy observed in oral lichen planus (OLP) and could function as a growth factor on the oral lichen planus (OLP)in a paracrine / autocrine fashion, activating intracellular pathways and ultimately leading cells to proliferate, avoid apoptosis or become insensitive.[27]

Immunohistochemical examination of syndecan-1 protein expression showed that neutralization treatment with anti-syndecan-1 protein accumulated around oral lichen planus (OLP) cells. Also, this indicates that syndecan-1 protein produced by cancer cells promotes their own invasion in an autocrine fashion and simultaneously promotes the proliferation of surrounding fibroblasts in paracrine fashion; thus, oral lichen planus (OLP) cells with higher invasion potential showed higher syndecan-1 protein expression, that implies that the level of syndecan-1 protein expression is indicator of degree of lichen planus (OLP) malignancy.[28]

Table 1
Syndecan-1 protein IHC expression in oral lichen plauns cases.

syndecan-1 protein core*	No.	%
1	4	13.3%
2	11	36.7%
3	15	50.0%
Total	30	100%

*1 (weak expression), 2 (moderate expression), 3 (strong expression)

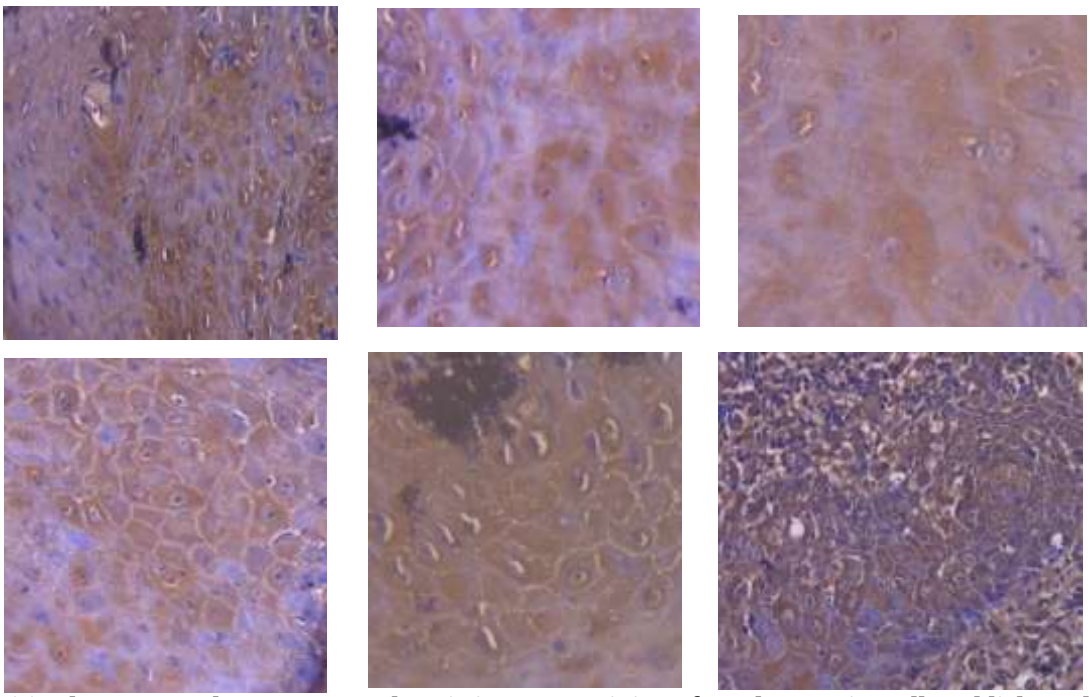

Figure 1: Positive brown membranous /cytoplasmic immunostaining of syndecan-1 in well oral lichen plauns (40X).

Table 2
p53expression in oral lichen plauns cases

p53 score*	No.	%
1	3	10.0%
2	9	30.0%
3	18	60.0%
Total	30	100%

*1 (weak expression), 2 (moderate expression), 3 (strong expression)

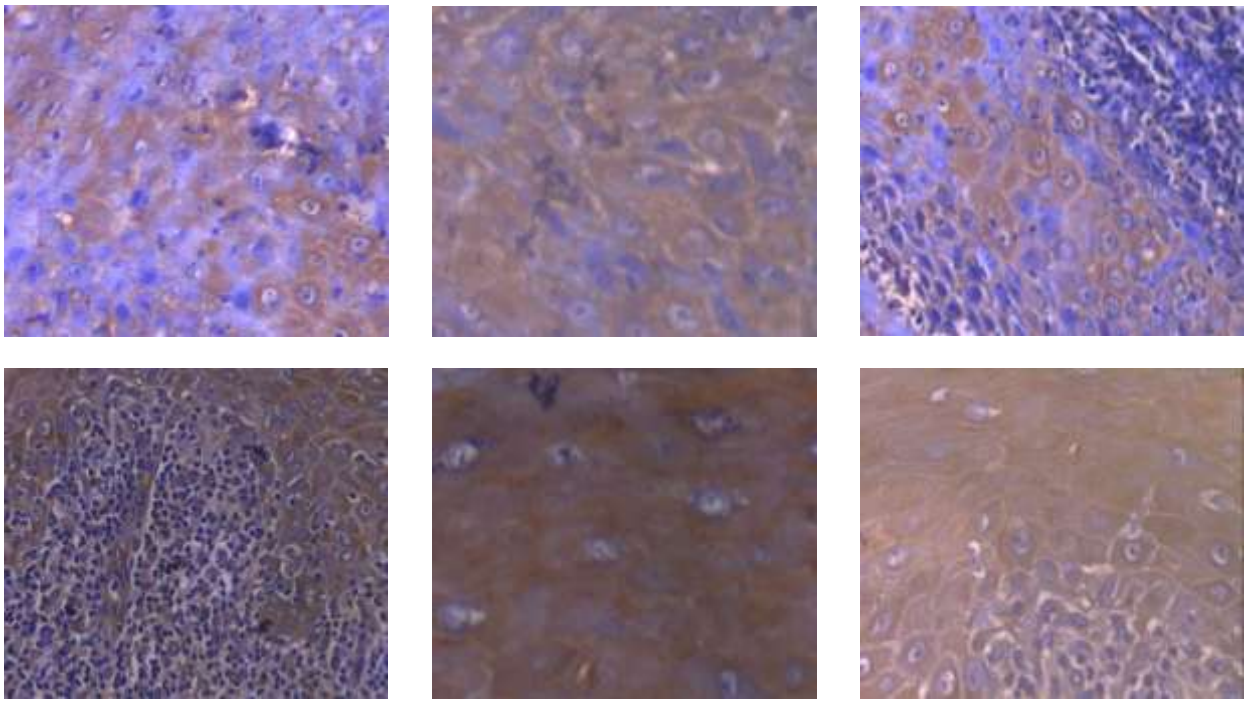

Figure 2: Positive brown nucleus immunostaining of P53 in moderate differentiated oral lichen plauns (OLP). (40X)

Table 3
The correlations between the IHC markers in the oral lichen plauns (OLP).

Marker	syndecan-1	*P53*	Endostatin
P53	Pearson Correlation	_	0.325
	Sig. (2-tailed)		0.001
	No.	30	30
syndecan-1	Pearson Correlation	0.325	_
	Sig. (2-tailed)	0.001	_
	No.	30	30

***P <0.001

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