Cell Surface Expression of 70 KDa Heat Shock Proteins and P21 in Normal Oral Mucosa, Oral Epithelial Dysplasia and Squamous Cell Carcinoma (An Immunohistochemical Study)

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ABSTRACT

Background: Oral SCC is a complex malignancy where environmental factors, viral infections and genetic alterations most likely interact, and thus give rise to the malignant condition. The HSP70 play a direct role in apoptosis inhibition by aligning the improved integrity of a cell's proteins with the improved chances of that particular cell's survival.P21 gene produces p21 protein which is a potent cyclin-dependent kinase inhibitor that plays a significant role in carcinogenesis. The aims of the study were to evaluate and compare the immun-histochemical expression of the HSP70 and cell cycle protein p21in NOM, OED, and OSCC. Correlate both marker expressions with each other.

Materials and methods: Forty six formalin-fixed, paraffin embedded tissue blocks(10 cases of normal oral mucosa,16 cases of oral epithelial dysplasia, and 20 cases of oral squamous cell carcinoma) were included in this study, an immunohistochemical staining was performed using antiHSP70monoclonalantibody, and anti p21 monoclonal antibody.

Results: Positive IHC expression ofHSP70 was found in 2 cases (20%) of NOM, 13 cases (81.3%) of OED and in 16 cases (85%) of OSCC. Positive IHC expression of P21 was detected In NOM in 2 cases (20%), while it was found in 9 cases (56.2%) of OED, and in 14 cases (70%) of OSCC .The difference between the expressions of both markers was statistically significant in NOM, highly significant in OED, and OSCC.

Conclusions: This study signify the important role of HSP70 and p21in oral carcinogenesis and in the evolution of the mucosa from normal to dysplastic to invasive carcinoma

Keywords: NOM, OED, OSCC, HSP70, p21. **(J Bagh Coll Dentistry 2016; 28(4):56-60)**

INTRODUCTION

Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by several genetic alterations. The use of several molecular biological techniques to diagnose oral precancerous lesions and cancer may markedly improve the early detection of alterations that are invisible under the microscope. This would identify patients at a high risk of developing oral cancer [1].

HSP70 regulates a wide range of protein-associated activities and elevated levels of HSP70 protect cells from apoptotic death. In OSCC, immune staining intensity for HSP70 is suggested to be related to the degree of tumor cell differentiation [2].

Cyclin-dependent kinase inhibitor, p21, the small 165 amino acid protein p21 (also known as p21WAF1/Cip1) mediates p53-dependent G1 growth arrest. Earlier studies supported the view that p21 suppresses tumours by promoting cell cycle arrest in response to various stimuli.

Additionally, substantial evidence from biochemical and genetic studies indicates that p21 acts as a master effector of multiple tumour suppressor pathways for promoting anti-proliferative activities that are independent of the classical p53 tumour suppressor pathway [3].

The lack of a unique marker of OSCC has long been a problem in the early detection of OSCC. It would be necessary to discover more reliable and efficient markers to characterize the malignant transformation of oral epithelium [4].

This study aimed to evaluate and compare the expression of HSP70 and p21 in normal oral mucosa, oral epithelial dysplasia, and oral squamous cell carcinoma, and to correlate both marker expressions with each other.

MATERIALS AND METHODS

The study samples included 46formalin-fixed, paraffin embedded tissue blocks (10 NOM, 16 OED, and 20 OSCC) dated from (1973 till 2013), obtained from the archives of the department of Oral and Maxillofacial Pathology/ College of Dentistry/ University of Baghdad; Al-Najaf Medical City. Sections of 4µm thickness were mounted on normal glass slides, stained with H and E and histopathologically re-evaluated. Histological grading for OSCC and oral epithelial

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dysplasia were recognized for each case according to WHO classification 2005 [5].

Fourother4µmthicksections for each case were cut and mounted on positively charged slides (Fisher scientific and Escho super frost plus, USA) for immunohistochemical staining with monoclonal antibody HSP70 using Abcam expose mouse and rabbit HRP/DAB immunohistochemical detection kit (Catalog No. Ab80436, Cambridge, UK), and monoclonal antibody p21 using Rabbit Anti- Human p21 antibody (Catalog No. A 181606) Dako Denmark immunohistochemical detection kit was used. HSP70 and p21 scoring system was according to Parvis and Faezah [6] and Mustafa et al. [7]

Statistical analysis

The study parameters were scored and considered as categorical data thus they presented as count and percentage, the relationship between categories was tested by Chi-square test.

Mann-Whitney U test was applied to assess the markers' comparison in each group as well as to assess groups' comparison in each marker. Pearson correlation was applied to assess the linear association between HSP70 and P21.

The level of significance was 0.05 (two-sided) in all statistical testing. The statistical analysis was performed using SPSS windows, version 19.

RESULTS

Positive HSP70Immunostaining was detected as brown cytoplasmic expression. (Figures 1, 2and 3)

IHC staining of HSP70 in NOM reveals that8 cases (80%) showed negative expression, 2 cases (20%) showed positive score I expression. And in OED, 3 cases (18.8%) showed negative expression, 3 cases (18.8%) showed score I positive expression, 9 cases (65.2%) showed score II positive expression, and 1 case (6.2%) showed score III positive expression. While in OSCC,IHC staining of HSP70 reveals that 3 cases (15.0%) showed negative expression, 2 cases(10%) showed score I positive expression, 13 cases(65%) showed score II positive expression, and2cases (10%) showed score III positive expression.

Positive p21 immunostaining was detected as brown (nuclear and cytoplasmic) expression. (Figures 4, 5and 6)

Regardingp21 expression in NOM, 8 cases (80%) showed negative expression and 2 cases (20%) showed positive expression. And in OED, 7cases (43.8%) showed negative expression, 4cases (25.0%) showed score I positive expression, and5 cases (31.2%) showed score II positive expression. While in OSCC, p21 immunostaining reveals that 6 cases (30%) showed negative expression, 8 cases (40%) showed score I positive expression, and 6 cases (30%) showed score II positive expression.

Regarding markers' (HSP70andP21 expression) comparison in each group and according to Mann-Whitney U test, the results revealed a statisticallya highly significant difference in NOM (p-value= 0.004), OED and OSCC (p=0.000), as clarified in table (1). Regarding groups' comparison in each marker, the results revealed statistically highly significant difference in HSP70 (p=0.001) and p21 (p-0.000) as clarified in table (2).

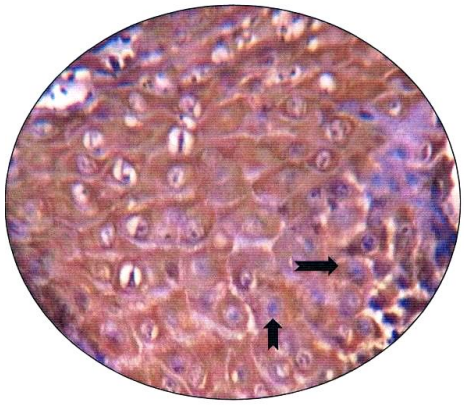

Figure 1: Positive cytoplasmic expression of HSP70 in mild dysplasia (400X)

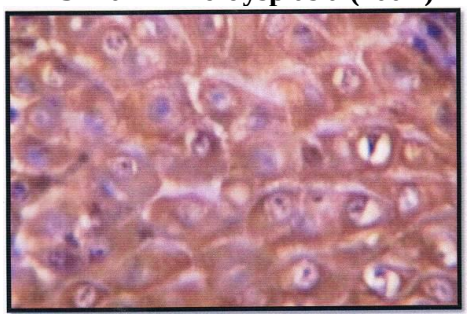

Figure 2: Positive cytoplasmic expression of HSP70 in moderate dysplasia (400X)

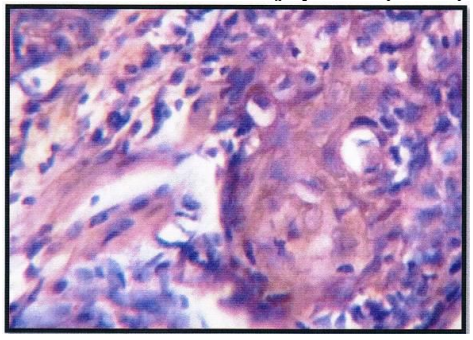

Figure 3: Positive cytoplasmic expression of HSP70 in well differentiated SCC (200X).

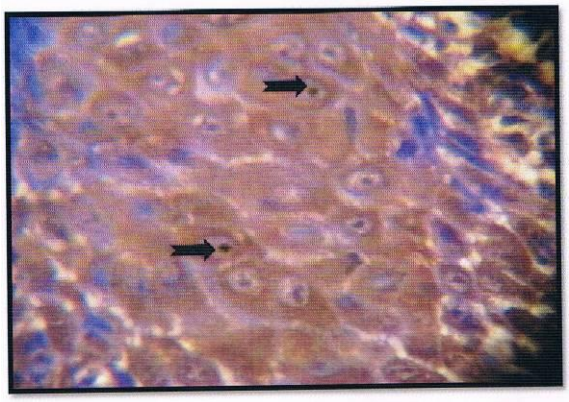

Figure 4: Positive nuclear and cytoplasmic expression of p21 in moderate dysplasia. (200X)

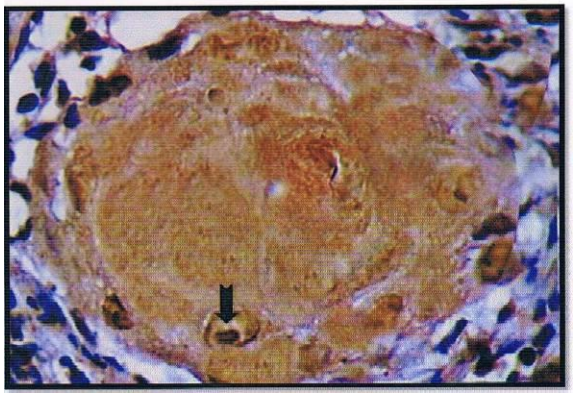

Figure 5: Positive nuclear and cytoplasmic expression of P21 in well differentiated SCC (400X).

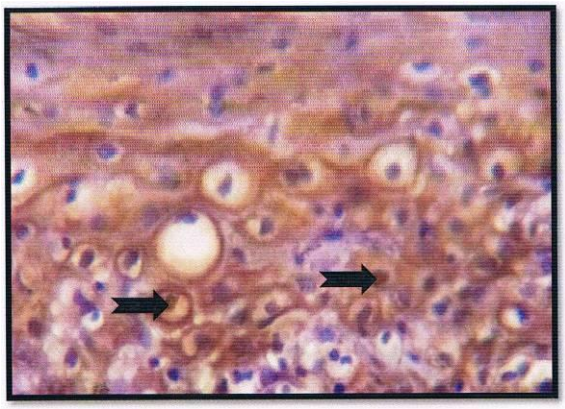

Figure 6: Positive nuclear and cytoplasmic expression of p21 in moderately differentiated SCC. (400X)

DISCUSSION

Assessment of HSP immunehistochemistry

The results of the present study showed that positive immune staining of HSP70 was found in 20% of normal oral mucosa cases. This result agrees with Seoane et al [8].

According to Nutan [9], the normal, non-diseased mucosa (free of any pathology/ source of irritation) of the oral cavity shows a faint expression of heat shock proteins in the epithelium.

Table 1: Descriptive statistics and markers' comparison in each group

Groups	Markers	Descriptive Statistics				Comparison	
		N	Mean	S.D.	S.E.	Mann-Whitney U test	p-value
NOM	HSP70	10	7.6	8.74	2.8	-2.81	0.004 (HS)
	P21	10	1.34	1.93	0.61		
OED	HSP70	16	22.1	15.1	3.8	-5.97	0.000 (HS)
	P21	16	6.1	6.23	1.56		
SCC	HSP70	20	31.5	16.7	3.7	-5.93	0.000 (HS)
	P21	20	6.68	6.52	1.46		

Table 2: Descriptive statistics and groups' comparison in each marker

Markers	Groups	Descriptive Statistics				Comparison	
		N	Mean	S.D.	S.E.	Mann-Whitney U test	p-value
HSP	NOM	10	7.6	8.74	2.8	13.07	0.001 (HS)
	ED	16	22.1	15.1	3.8		
	SCC	20	31.5	16.7	3.7		
P21	NOM	10	1.34	1.93	0.61	23.67	0.000 (HS)
	ED	16	6.1	6.23	1.56		
	SCC	25	8.68	6.52	1.46		

Concerning OED cases the results of this study showed that positive expression of HSP70was observed in (81.8%) of OED cases.

HSP positivity was found in (85%) of OSCC cases.

Over expression of HSP70 in oral cells may reflect a state of biological stress experienced by premalignant and malignant cells. Alternatively, high levels of HSP70 may be a requirement, or may be associated with a state of increased cellular activity or cell proliferation [8]

Sugerman et al. [10] studied (HSP70) expression in OSCC, epithelial dysplasias and benign oral mucosal lesions by comparing their staining intensity. Median staining intensity was significantly greater in SCC, epithelial dysplasias and benign oral mucosal lesions compared to normal mucosa. However, staining intensity in poorly differentiated squamous cell carcinoma was greater than that in moderately differentiated SCC, though no statistical significance was observed.

Assessment of p21 immuno histochemistry

The present study showed positive p21 immuno-reactive in NOM in (20%) of cases, And in OED it was found in (56.%). These results were in agreement with Huang et al [11] that's showed positive p21expression in NOM and OED, According to clinic-pathological correlation of P21 and OED, the results of this study showed statistically non-significant correlation, which agrees with Choi et al [12].

P21 positivity was found in (70%) of OSCC cases. Agree with Yuen et al [13]. That showed higher P21 expression in the OSCC group.

In the dysplastic epithelium, p21 increases its expression as the degree of dysplasia increases. In OSCC, expression is variable, especially in poorly differentiated tumor areas [14].

It has been well documented that alterations of levels of p21 expression are early events in the development of dysplastic oral epithelial cells and lingual carcinoma [15].

The present study showed statistically significant difference between HSP70 andP21 in NOM, and highly significant difference between them in OED and OSCC.

Correlation between HSP70 and P21 in each group

This is the first study in Iraq and other parts in the world assessing the correlation between HSP 70 and P21immunohistochemical expression in NOM, OED, and OSCC. Since this is a pioneer research in assessing that correlation, so the comparison could be withdrawn from other studies using other tissue specimen, which is in agreement with Malusecka et al [16] that showed there was significant correlation between HSP70 and P21 in lung cancer.

The present study showed ahighly significant correlation in HSP70 and p21 regarding groups' comparison. This means that HSP70 andP21 play a role in oral carcinogenesis.

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