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Characteristic Intensity And pattern Of Staining Of P53 And Ki67 In Oral Squamous Cell Carcinoma

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Abstract

To investigate the characteristic intensity and pattern of staining of P53 and Ki67 in oral squamous cell carcinoma in different grading of lesions.

Thirty patients with oral squamous cell carcinoma were collected and used to detect the intensity and pattern of staining of P53 and ki67 markers by immunohistochemical method.

Immunoreactivity of this study for P53 found that 21 (70%) were positive in different grading of disease. Most frequent combination includes strong intensity in 11 cases (52%) and diffuse pattern in 15 cases (71%).

Immunoreactivity of Ki67 in oral squamous cell carcinoma indifferent grading was found positivity for ki67 in 25 cases (83%) and the most frequent pattern of staining was the scattered which seen in 20 cases (80%) and 5 cases (20%) had diffuse pattern of staining.

In conclusion the study revealed that the intensity and pattern of staining of P53 and Ki67 correlate with well differentiated oral squamous cell carcinoma.

Keywords: Oral squamous cell carcinoma, P53, Ki67, Intensity and pattern of staining.

Introduction

Oral cancer is a world health problem; estimates indicate that there are over 390000 new cases diagnosed each year of which 4400 occur in the UK[1]. Patients with oral cancer have a poor prognosis and typically, more than a third of the patients die within 5 years of diagnosis[2]. Such poor survival has been attributed to the late presentation of the disease, the high recurrence rate at the site of the original tumor and the development of second primary tumours[3, 4].

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy and the commonest malignant tumor of the oral cavity, accounting for more than 90% of all mouth malignancies. Oral squamous cell carcinoma usually afflicts middle-to older aged patients who have been chronic users of tobacco and alcohol[5].

Oral carcinogenesis is a multistep process in which 6-10 genetic events lead to the disruption of the normal regulatory pathways that control basic cellular functions including cell division, differentiation, and cell death[6]. A series of genetic alterations of both oncogenes and tumor suppressor genes are important steps in oral carcinogenesis. The P53 gene, a well-known tumor suppressor gene, is believed to serve as a gate keeper against carcinogenesis:-

1. Under normal circumstances, the function of P53 protein is to prevent the propagation of genetically damaged cells.
2. P53 assists in DNA repair by causing G1 arrest and inducing DNA repair genes or direct apoptosis in cells which are genetically damaged beyond repair[7].

To date, alteration of P53 gene are the most common event in human cancers, including oral squamous cell carcinoma[8].

Ki67 is a nuclear non-histone protein expressed maximally in cells in G2 and M phases of cell cycle, but absent in resting cells[9]. Ki67 can be employed to measure the growth fraction of normal tissues and malignant tumor, so Ki67 can be used as a prognostic marker for oral squamous cell carcinoma[10].

The aim of this study is to investigate the intensity and pattern of staining of tumour suppressor gene (P53) and cell proliferation marker (Ki67) in oral squamous cell carcinoma in different grading of disease.

Material and Methods

Thirty patients with oral squamous cell carcinoma were collected from Maxillofacial Center in Specialized Surgery Hospital Baghdad Iraq and Oral Pathology Department, College of Dentistry, University of Baghdad were used to detect Intensity and pattern of staining of P53 and Ki67 in different grading of disease by immunohistochemical method.

The material was analyzed using immunohistochemical staining P53 expression was assessed using P53 (D0-7) antibody (Dako, Denmark).

Ki67 (MIB-1) antibody (Dako, Denmark) was used to assess cell proliferation. Briefly, 5µm section were cut and mounted on the positive charged glass slides. Sections were dewaxed in xylene and rehydrated in graded ethanol, then the sections were pretreated with antigen retrieval solution (citrate buffer PH 6.0), the sections then rinsed in phosphate buffer saline (PBS). Endogenous peroxidase activity was blocked with enough hydrogen peroxide then enough primary antibody were applied to the sections incubated at 37ºc for 15 minutes, in the next day the detection system employed a biotinylated secondary antibody applied to section and incubated at 37ºc for one hour. Then one to two drops of streptavidin-HRD reagent applied to sections incubated at 37ºc for 30 minutes. Then enough drops of chromogen reagent applied to sections incubated for 10 minutes at 37ºc. Sections were counterstained with Mayer's haematoxylin stain for 30 second then the sections were dehydrated with graded ethanol and xylene and then mounting with Dpx mounting media.

Evaluation of Immunohistochemical result of P53 staining:-

1. Positive P53 protein expression give clear cut nuclear brownish staining.
2. Intensity of staining of brownish coloration was consider strong if could be detected very cleary at even low magnification (10x) and moderate if it was detected with difficulty at low magnification, and weak if it was detected only at high magnification, table (1).

Ki67 immunohistochemical result: any nuclear staining of Ki67 regardless of its intensity was considered to be positive.

The pattern of staining for P53 and Ki67 was consider diffuse if the positive cells were distributed through

almost all the sections while it was consider patchy if more than one area of the section showed large number of positive cell and if they were only very few cells positive in the section then a scattered pattern was considered. Table (2), figure (1).

Result

A total of thirty patients with oral squamous cell carcinoma were included in this study, their ages range between 45-85 years.

Immunoreactivity for P53 in 30 cases of oral squamous cell carcinoma in different grading of disease shows the positive nuclear brown staining detected in 21 cases (70%)out of 30 cases, 14 cases (67%) were well differentiated oral squamous cell carcinoma, 4 cases (19%) were moderately differentiated and 3 cases (14%) were poorly differentiated oral squamous cell carcinoma table (2), while 9 cases (30%) was considered as negative staining for P53 figure (2).

The most frequent combination included strong intensity in 11 cases (52%) and diffuse pattern in 15 cases (71%). Table (3), figure (3).

Seven cases ((33%) had strong intensity and diffuse pattern of staining, seven cases (33%) had moderate intensity and diffuse pattern of staining, three cases (14%) had strong and scattered pattern of staining, only one case (5%) had strong intensity with patchy pattern of staining, table (4), figure (3).

Interpretation of P53 positivity:

Intensity and pattern of nuclear staining were examined and specified for each of 21 P53 positive cases.

The intensity was strong in 11 cases (52%). Moderate in 9 cases (43%) and weak in one case only (5%).

The most frequent pattern was diffuse staining which seen in 15 cases (71%) table (3).

There was no significant associations between the intensity of P53 immunostaining and different histological grades of oral squamous cell carcinoma as shown in table (5).

Similarly, there were statistically no significant differences observed in the pattern of P53 protein staining in different histological grades of oral squamous cell carcinoma, Table (6).

Ki67 immunoreactivity was clearly evident as diffuse or dot like nuclear staining with nucleolar accentuation making it easy to decide whether a cell was positive or not.

In the current study, positivity for Ki67 was demonstrated in 25 cases (83%) out of 30 cases, 17 cases (68%) were well differentiated oral squamous cell carcinoma.5 cases (20%) were moderately differentiated and 3 cases (12%) were poorly differentiated oral squamous cell carcinoma and only 5 cases (17%) were negative for Ki67, figure (4).

Interpretation of ki67:

The most frequent pattern was the scattered which was seen in 20 cases (80%), 5 cases (20%) had diffuse pattern of staining, table (7), figure (5).

Statistically not significant difference was observed between the pattern of Ki67 immunostaining and histological grade of oral squamous cell carcinoma, table (8).

Discussion

The significance of the apoptotic pathway in the development and progression of human malignant tumours has become a major topic of discussion during the last years[11]. Mutations in the P53gene are the most frequent mutations encountered in human tumours[12, 13].

The roles of P53 as prognostic indicators have been widely investigated in oral cancers and precancerous lesions[14-16]. In the present study strong intensity and diffuse pattern of P53 staining was seen in 11 cases and 15 cases respectively while weak intensity and patchy pattern only seen in one case and this is controversial with other study[17].

Statistical analysis revealed no significant differences between intensity of staining with histological grade of oral squamous cell carcinoma, therefore it is reasonable to conclude that strong/Diffuse staining detected in well differentiated oral squamous cell carcinoma and the biological significance of the staining remain unclear.

Ki67 is an antibody that reacts with a nuclear non-histone protein being present in all active parts of the cell cycle and can be used to estimate the growth fraction of tumours[18, 19]. Ki67 shows the proliferation capacity of cancer cells regardless intensity of staining.

The present study reveals that high percentage of proliferating cells with scattered distribution in well differentiated oral squamous cell carcinoma and this is controversial with other study[20].

Statistical analysis revealed no significant differences between patterns of staining with the histological grade of oral squamous cell carcinoma.

In summary, tumor suppressor gene (P53) and cell proliferation marker (Ki67) expressed in oral squamous cell carcinoma. Strong/Diffuse pattern of P53 staining and scattered pattern of Ki67 staining correlate with well differentiated oral squamous cell carcinoma.

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Table (1): Interpretation of P53 positivity.

Interpretation	Intensity staining
Weak(w)	Barely detectable
Moderate (M)	Detectable (neither weak nor strong)
Strong (S)	Very dark brown

Table (2): pattern of staining of P53 and Ki67 positivity.

Interpretation	Pattern of staining
Diffuse (D)	Homogenous distribution in all section
Patchy (P)	Patchy positivity
Scattered (S)	Very few positive cell in section

Table (3): Characteristics of P53 positive staining.

Interpretation		N (%)
Intensity	strong	11 (52%)
	Moderate	9 (43%)
	weak	1 (5%)
Pattern	Diffuse	15 (71%)
	Scattered	5 (24%)
	patch	1 (5%)

Table (4): Variable combination of intensity and pattern of staining of P53positivity.

Positivity	N (%)
Strong and diffuse	7 (33%)
Strong and scattered	3 (14%)
Moderate and diffuse	7 (33%)
Moderate and scattered	2 (10%)
Weak and diffuse	1 (5%)
Strong and patch	1(5%)

Table (5): comparison between the intensity of P53 and histological grade of oral squamous cell carcinoma.

Histological grade	Intensity of P53			Total N (%)	P
	Strong N (%)	Moderate N (%)	Weak N (%)		
Well differentiated	7 (50%)	6 (43%)	1 (7%)	14 (67%)	NS
Moderately differentiated	0 (0%)	4 (100%)	0 (0%)	4 (19%)	
Poorly differentiated	1 (33%)	2 (67%)	0 (0%)	3 (14%)	
Total N (%)	8 (38%)	12 (57%)	1 (5%)	21 (100%)	

NS not significant, P >0.05

Table (6): comparison between the pattern of P53 immunostaining and the histological grade of oral squamous cell carcinoma.

Histological grade	Diffuse N (%)	Scattered N (%)	Patchy N (%)	Total N (%)	P
Well differentiated	10 (71%)	3 (21%)	1 (8%)	14 (67%)	NS
Moderately differentiated	3 (75%)	1 (25%)	0 (0%)	4 (19%)	
Poorly differentiated	2 (67%)	1 (33%)	0 (0%)	3 (14%)	
Total N (%)	15 (71%)	5 (24%)	1 (5%)	21 (100%)	

NS not significant,P >0.05

Table (7): Interpretation pattern of staining of Ki67 in oral squamous cell carcinoma.

Interpretation		N (%)
Pattern	Scattered	20 (80%)
	Diffuse	5 (20%)
	Patchy	0 (0%)

Table (8): The pattern of Ki67 in different histological grades of oral squamous cell carcinoma.

Histological grade	Diffuse N (%)	Scattered N (%)	Patchy N (%)	Total N (%)	P
Well differentiated	3 (18%)	14 (82%)	0 (0%)	17 (68%)	NS
Moderately differentiated	2 (40%)	3 (60%)	0 (0%)	5 (20%)	
Poorly differentiated	0 (0%)	3 (100%)	0 (0%)	3 (12%)	
Total N (%)	5 (20%)	20 (80%)	0 (0%)	25 (100%)	

NS not significant,p>0.05

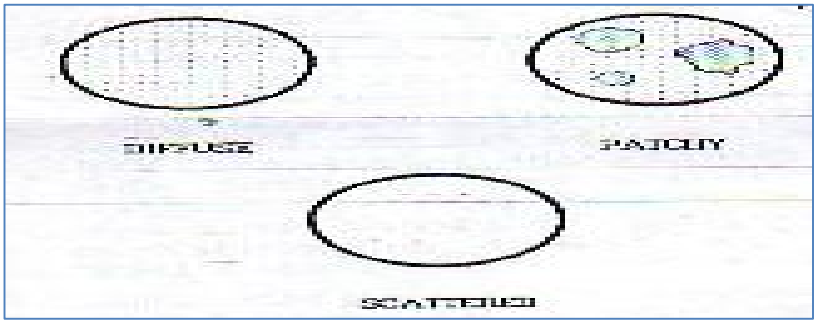

Figure (1): The distribution patterns of P53 and Ki67 positive cells.

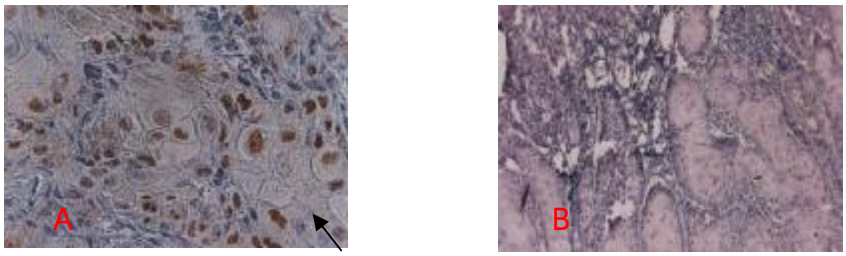

Figure (2): Immunohistochemical staining for P53. (A) OSCC (well differentiated) positive nuclear staining (X200). (B) OSCC (well differentiated) negative staining for P53.

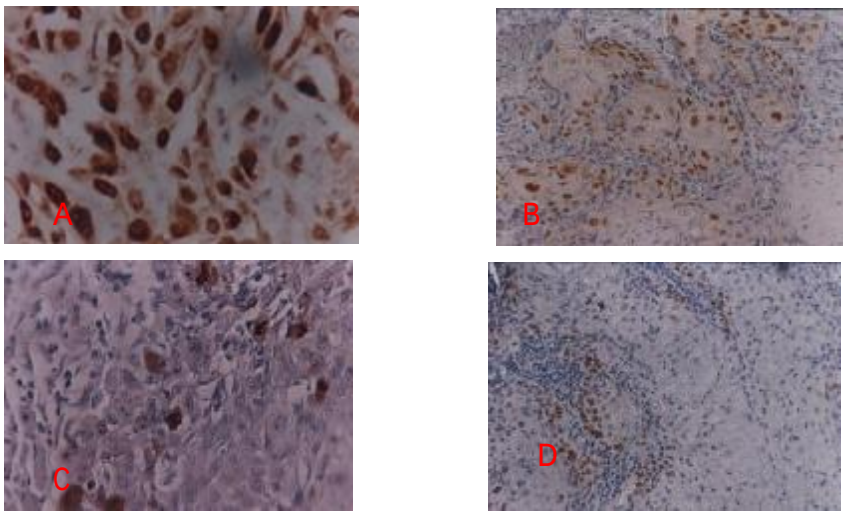

Figure (3): Immunohistochemical staining for P53. (A) OSCC (poorly differentiated) showing strong intensity and diffuse pattern(X200). (B) OSCC (well differentiated) showing moderate intensity and diffuse pattern (X400). (C) OSCC (well differentiated) showing strong intensity and scattered pattern (X400). (D) OSCC (well differentiated) showing strong intensity with patchy pattern (X200).

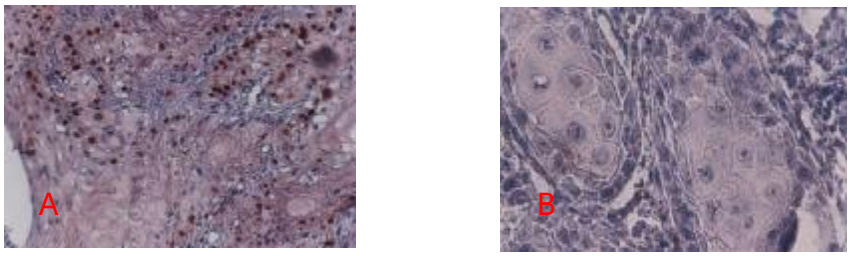

Figure (4): Immunohistochemical staining for Ki67. (A) OSCC (well differentiated) showing positive nuclear staining (X200). (B) negative staining for Ki67 (X400).

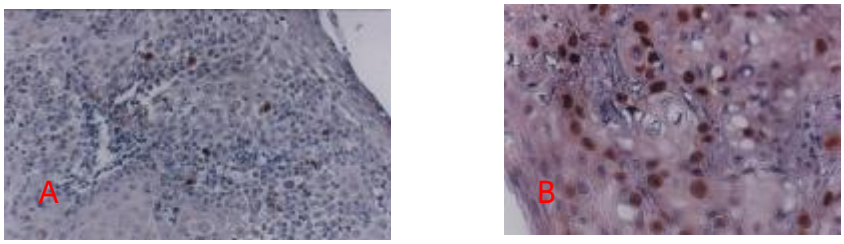

Figure (5): Immunohistochemical staining for Ki67. (A) OSCC (well differentiated) showing scattered pattern of staining (X200). (B) OSCC (well differentiated) showing diffuse pattern of staining (X400)