الجين P53 كدالة في التطور السلوكي لسرطان الفم الحرشفي

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الملخص

خلفية البحث: يعدُّ الجين P53 جين مثبط لنمو الأورام الخبيثة ولكن عند إصابته بأي خلل يفقد وظيفته، وعليه تزداد نسبة حدوث هذه الأورام.

رغم كل التطور الذي شهده الطب في السنوات الأخيرة يبقى سرطان الفم الحرشفي من أخطر الأنواع وأكثرها حدوثاً، وعليه تبقى الحاجة للعمل على أي دالة تساعدنا في معرفة نمو هذا الورم وسلوكه.

هدف البحث: للكشف عن وجود علاقة بين وجود بروتين الجين P53 ودرجة التميز النسيجي لسرطان الفم الحرشفي.

المواد والطرائق: أُجْرِيَتْ هذه الدراسة على 30 مقطعاً لسرطان الفم الحرشفي خلال الأعوام (2001 – 2004) في أرشيف قسم أمراض الفم/ جامعة بغداد، واستخدمت ثلاثة مقاطع من بطانة الفم الطبيعية للمقارنة السلبية، وثلاثة مقاطع من سرطان الثدي للمقارنة الإيجابية من دائرة المختبرات التعليمية/ مدينة الطب في بغداد.

النتائج: أظهرت نتائج هذه الدراسة علاقة وثيقة بين وجود بروتين الجين P53 والتميز النسيجي لسرطان الفم الحرشفي حيث ظهر البروتين بنسبة40%في سرطان الفم الحرشفي جيد التمايز وبنسبة80%للحالات المتوسطة التمايز، في حين كانت النسبة100% في حالة سرطان الفم الحرشفي الضعيف التمايز، وهذا يساعد في التكهن بسير المرض المستقبلي كون الورم نتج عن طفرة وراثية للجين P53 والذي يختلف في سيره المستقبلي، وحتى في استجابته للعلاج فيما بعد عن الورم الذي لا يظهر فيه مثل هذا التغير.

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P53 as an Indicator for the Prognosis of Oral Squamous Cell Carcinoma

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Abstract

P53 is a tumor suppressor gene, when it is mutated it loss it's function. This study was conducted to evaluate the detection of the p53 protein in oral squamous cell carcinoma with different histological behavior and it was found that the poorly differentiated S.C.C. recorded the highest mutated type so; p53 protein detection may help in studying the prognosis of the tumor and it's response to the treatment.

Key words: p53 in Oral Cancer, Oral Squamous Cell Carcinoma

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Introduction

P 53 is a tumor suppressor gene, which plays a key role in cell cycle arrest, and induction of apoptosis in genetically damaged cell.

In spite of the marked improvement in the treatment modalities available for oncologists of the head and neck tumor, still there is a high mortality rate, which is about 30% within five years of diagnosis [(1).] This necessitate the need for early detection and diagnosis of oral cancer as well as the need for factors that may have a prognostic or predictive value and reflect biologic behavior of a malignant lesion [(2).] Squamous cell carcinoma is the most common malignant neoplasm in the oral cavity, representing about 90% of all oral cancers [(3, 4).] The use of biologic markers to screen patients who are at increased risk may help to predict the probability of disease progression, aid in the diagnosis made by routine histopathologic studies, assess the prognosis of the individual cancer patient, develop treatment protocols and evaluate the response to therapeutic agents [(5,6)].

Oral squamous cell carcinoma can be defined as a malignant neoplasm of stratified squamous epithelium that is capable of locally destructive growth and distant metastasis [(7, 8).] Although it may occur in various oral sites, it is most common on the lower lip, lateral borders of the tongue and the floor of the mouth. The incidence of squamous cell carcinoma increases with age, most of which occur after 50 years of age in men, although the relative incidence among women and younger patients seems to be increasing [(9).] Histopathologically it can be graded into well differentiated squamous cell carcinoma in which tumor tissue closely resembles its tissue of origin, the neoplasm is obviously squamous in type, and consists of masses of prickle cell with limiting layers of basal and keratin pearls formation and moderately differentiated squamous cell carcinoma in which the keratin pearls are sparse or absent, and the prickle cells and their nuclei are much more pleomorphic. ***Finally***, poorly differentiated, anaplasia in which the malignant cells
are even more irregular and may hardly be recognizable as epithelial cells, no keratinization could be detected [(10).]

Aim of the Study:

To detect the association between P53 positivity and histological grade progression of oral squamous cell carcinoma (O.S.C.C.).

Materials and Method:

This study was conducted on thirty sections of oral squamous cell carcinoma during the period (2001-2004) from the archive paraffin blocks in the department of Oral Pathology in the Faculty of Dentistry, University of Baghdad.

Three negative control blocks of normal oral epithelium were taken from the same archive, while three positive control blocks of a tissue known to contain the target antigen against which the primary antibody raised. In this study the tissue was ductal breast carcinoma which was obtained from the Teaching Laboratories in the Medical City. The tissues had been fixed in 10% neutral buffered formalin. From each of the thirty paraffin-embedded blocks two tissue sections were cut at 5μm, one used for histopathological diagnosis and the other for immunohistochemical evaluation, so they were mounted on probe-on plus slides (Fischer brand), then sections were immersed in retrieval solution and heated in water bath at 95 C^{o} for 30 minutes, the sections then were cooled at room temperature for 20 minutes to rinse them in phosphates buffer saline and bathing for 3 minutes the endogenous peroxidase activity was blocked by incubating the sections in hydrogen peroxide (4-6) drops for 7 minutes at room temperature, followed by immersing the sections in PBS (Phosphate-buffered solution) for 3 minutes, then excess solution was removed by blotting off the slides, the non-specific primary and secondary antibody binding was inhibited by incubating the sections with power block reagents for

7 minutes at room temperatures, then excess solution was blotted off. We have tried not to rinse the sections, then 0.05-0.20 ml (4-6) drops of the primary antibody were appropriately diluted and sections were incubated for 45 minutes at room temperature

and rinsed well with PBS for 10 minutes and wiped around the sections. This step was omitted in negative control slides.

Then (4-6) drops of diluted biotinylated goat-anti-mouse IgG (link) solution were added, incubating for 20 minutes at room temperature and rinsed well with PBS for 10 minutes and wiped around the sections. After this (4-6) drops of diluted peroxidase labeled streptavidin (label) solution were added (mixed and diluted appropriately at least 30 minutes before use). Incubated for 20 minutes at room temperature and rinsed well with PBS for 2-5 minutes, then chromogen solution was applied, slides were

incubated for 20 minutes at room temperature, or until desired color intensity had been reached, rinsed well with tap water for 2 minutes, then sections were counter stained in Harris's hematoxylin solution for 7 minutes, rinsed and dehydrated. While negative control sections were incubated with PBS instead of primary antibody; ductal breast carcinoma sections were served as positive controls. The above – mentioned IHC procedures were all done in teaching – Laboratories – Medical City of Baghdad (according to instruction of the Immunotech, Marseille, Codex-9-France).

Evaluation of IHC stained sections:-

Whole tissue sections were examined with light microscope for P53-positive nuclear staining at x 100 and at x 400. The P53 expression was recorded as positive if > 10% of all the tumor cells positive for nuclear P53 staining and as negative if < 10% were positive cells based on Ibrahim and Johannessen method which depend on the visual evaluation of the field. [11].

Results:

These results are based on the analysis of histopathologyical and immunohistochemical findings of 30 cases of oral squamous cell carcinoma. Based on the Cotran et.al. (1999) the histopathological grading system is related to biological behavior of the tumor cells; our study detected that out of 30 samples, 15(50%) fig. 1-A, 5(16.6%) and 10 samples
(33.3%) fig. 1-B were well, moderately and poorly differentiated oral squamous cell carcinoma respectively(table I).

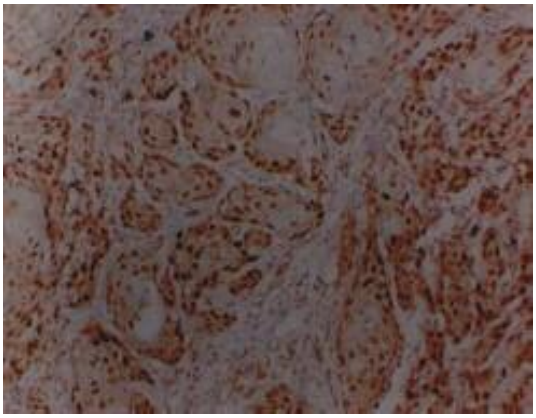

Fig. 1-A
Well differentiated OSCC x 100
H & E stain
Mass of prickle cells with Keratin Pearls

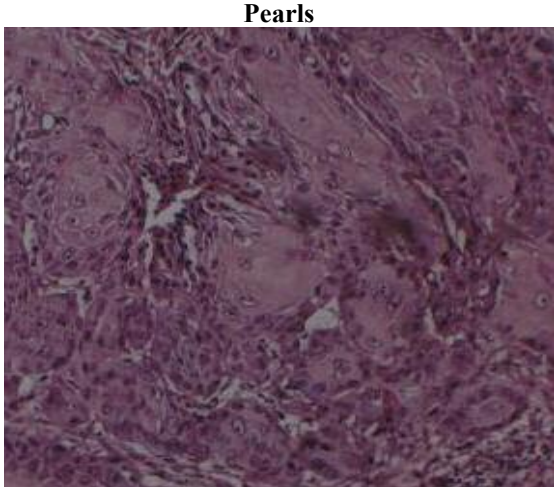

Fig. 1-B
Well differentiated OSCC x 100
P53 appearance in immunohistochemistry
Brown stained nuclei result from condensed mutated P53 protein

Table (I) Histopathological Types of Oral Squamous Cell Carcinoma and Their Percentage

Histopathological Differentiation	No. of samples	%
well	15	50
moderate	5	16.66
poor	10	33.33
Total	30	100%

Regarding the P53 positivity, our study found that only 6 (40%) fig. 2-A out of 15 samples of well differentiated OSCC, 4 (80%) out of 5 samples of moderately differentiated OSCC and all samples of poorly differentiated OSCC were positive (100%) fig. 2-B (table II).

Table (II) Immunohitochemical Positivity of P53 in Oral Squamous Cell Carcinoma

No. of Cases	Positive Cases	Percent
15	6	40
5	4	80
10	10	100

On the other hand no one from the three normal oral squamous epithelia sections (control) showed P53 positivity.

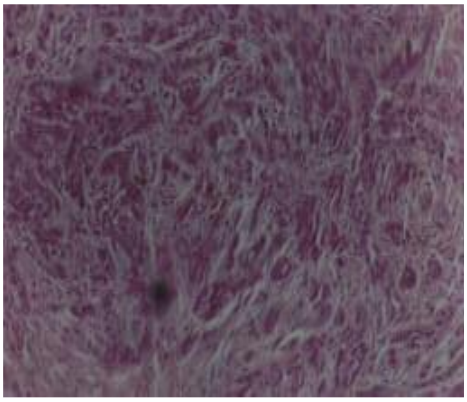

Fig. 2-A
Poorly differentiated OSCC x 100
H & E stain
Malignant cells are more irregular and hardly be recognizable as squamous epithelial cells.

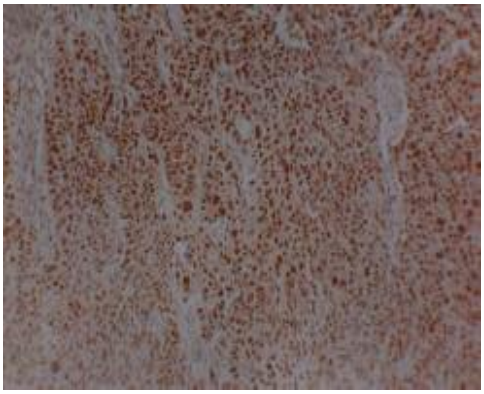

Fig. 2-B
Poorly differentiated OSCC x 100
P53 appearance in immunohistochemistry
Dark brown nuclei result from condensed mutated P53 protein in highly progressive Tumor.

Discussion:

The results of our study demonstrated P53 expression in well, moderate and poorly differentiated squamous cell carcinomas. Regarding P53 detection, stabilization of the mutant P53 protein allows IHC to be routinely used to demonstrate the mutant P53 protein in tissue samples. However, normal P53 protein is undetectable by this technique due to it's short half life.[12,13,14] The Positivity of P53 detection was observed in 40% of well differentiated squamous cell carcinomas and 80% in moderately differentiated squanmous cell, and 100% in poorly differentiated this observation is similar to several studies in the world which showed higher levels of mutant P53 of the tumor cells in comparison with mature cells. P53 expression strongly associated with DNA ploidy and measures of proliferation and nuclear grade. [15,16].

In other types of cancers, such as medullary carcinoma, it was found that carcinomas with poorly differentiated histological grade were more P53 positive [17, 18]. It may indicate that P53 positivity seems to correlate strongly with histological progression of the disease, which confirms the importance of P53 alterations in oral carcinogenesis. This finding is explained on the basis that there is an inconsistent relationship between gene mutations and the level of P53 protein staining by IHC which seems to occur relatively late and are associated with trans, formation to the invasive phenotype [19, 20, 21, 22].

Conclusion & Recommendations:-

Our study detected direct relationship between oral squamous cell carcinoma histological behavior and P53 positivity from well differentiated 40%to poorly differentiated 100%. Therefore, we recommend the use of P53 as an indicator for the prognosis of OSCC because we know that poorly differentiated type is of bad prognosis specially when it is result from genetic alterations such as (p53 mutation).

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