# Expression of P63 Gene in Oral Squamous Cell Carcinoma

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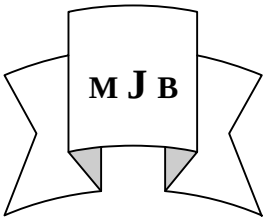



## Abstract

The present study was conducted on oral squamous cell carcinoma(OSCC)which is the most common oral malignancies with main objective of showing the expression of p63 in OSCC.

This study comprised a total of (93) patients diagnosed as OSCC.,(62)males and (31) femalesThe cases include: well differentiated SCC.(32)cases, moderately differentiated SCC.(40) and poorly differentiated SCC.(21)cases.The expression of p63 gene was carried out on 4µm tumor sections using Immuno histochemical staining of p63 antibody.

The data demonstrated the mean percentage expression of p63 in well, moderately and poorly differentiated SCC.Results showed that p63 expression was observed as positive diffuse reactivity in(38) cases (40.86%), positive basal Para basal reactivity in (19)cases (20.34%), positive basal reactivity in (34) cases (36.56%) and negative reactivity in(2)cases (2.15%)

Results also showed p63 expression was increased in high grade tumors of size >1.5cm and metastatic dissemination of lymph nodes with diffuse and basal Para basal pattern of distribution.Scoring analysis showed more than 50% of cases present with score 26-50% of expression.

In conclusion , these data found that the P63 nuclear positivity was extended from the basal- Para basal layers to the whole epithelial layers in both moderately and poorly differentiated tumors , and P63 staining was more uniform and homogenous for the well- differentiated tumor areas.

## الخلاصة :

إن العمل الراهن يتناول دراسة عن سرطان الفم الحرشفي الذي يعتبر من أكثر أنواع أمراض سرطان الفم شيوعا ويهدف بصورة أساسية للكشف عن مدى تعبير ألجين p63 في حالات سرطان الفم الحرشفي .

هذه الدراسة شملت (٩٣) مريضا شخصوا بإصابتهم بسرطان الفم الحرشفي ،(٦٢) ذكور و(٣١) إناث وبحالات مختلفة، إلى سرطان حرشفي حسن(٣٢) حالة وسرطان حرشفي وسط (٤٠) وسرطان حرشفي سيئ (٢١) .

لقد تم تقييم مدى التعبير عن ألجين في مختلف حالات سرطان الفم الحرشفي باستخدام الصبغة الكيميائية النسجية المناعية للبروتين p63 الذي ينتجه ألجين p63 وهو جين يمنع تكوين الأورام في الجسم وتمت الصبغة على شرائح سرطانية بسمك (٤) مايكرو متر .

كما تم حساب الوسط الحسابي لمدى التعبير عن هذا ألجين في مختلف طبقات بطانة الفم .

أظهرت النتائج إن التعبير عن هذا ألجين كان منتشرا في كافة طبقات بطانة الفم في (٣٨) حالة (٤٠،٨٦%) ومنتشر في الطبقة الأساسية وقبل الأساسية في (١٩) حالة (٢٠،٤٣ %) ومنتشر في الطبقة الأساسية فقط في (٣٤) حالة (٣٦،٥٦ %) ولا يوجد تعبير في حالتين فقط (٢،١٥ %) .كما أظهرت النتائج إن التعبير عن ألجين p63 يزداد في الأورام ذات التمييز السيئ وبحجم أكثر من ١،٥ سم ومنتشر إلى الغدد اللمفاوية وبتوزيع مختلف في طبقات

بطانة الفم .مما سبق نستنتج إن التعبير عن ألجين p63 يمتد من الطبقة الأساسية وقبل الأساسية والى كافة الطبقات في كلا النوعين من سرطان الفم الحرشفي الوسط والسيئ وان صبغة p63 تكون أكثر متجانسة ومنتظمة في سرطان الفم الحرشفي الحسن .

## Introduction

P63 a member of the P53 tumor suppressor family of genes is vital for normal epithelial development and may play a critical role in epithelial tumor formation (10). These proteins are thought to act as transcription factors that regulate the progression of the cell through its cell cycle and cell death (apoptosis) in response to environmental stimuli, such as DNA damage

P63 gene encodes at least six different proteins with homology to the tumor suppressor protein p53 and the related p53 family member p73 so far there have been limited data concerning the expression patterns of individual p63 , due to a lack of reagents that distinguish between the different isoforms (15).

P53 tumor suppressor protein plays a critical role in the regulation of the cell cycle and apoptosis. Recently two p53 related proteins p73 and p63 were identified as members of the p53 gene family (1)
Abnormalities in the p53 gene are regarded as the most consistent genetic abnormalities detected in head and neck SCC.(HNSCC).(5)
Two related members of the p53 gene family , p73 at the 1p36 region and p63 at the 3q 27-29 region have shown remarkable structural similarity to p53 suggesting possible functional and biological interactions (8).

They share considerable sequence homology with p53 in the transactivation, DNA binding and oligomerization domains indicating possible involvement in carcinogenesis (4).

P63 is expressed as three major forms, p63 alpha, p63 beta and p63 gamma, each of which differ in their c- termini (1). All three forms can be alternatively transcribed from a cryptic promoter located within intron 3, producing delta N p63 alpha, Delta Np63 beta, and hypoxia(12). The P63 protein is expressed in the nucleus of basal cells in many types of epithelium (14). The human p63 gene encodes a series of protein isoforms that differ in their N and /or C- terminal sequences and possess varying activities in promoting or repressing p53 related functions and in regulating the proliferation and differentiation of epithelial cells (6).
and Delta Np63 gamma. The high degree of similarity of p73 and p63 to evolutionarily conserved regions of p53 suggests that these proteins play an important role in
regulating cell cycle arrest and apoptosis.(1).
p63 is essential for normal epithelial development and is over expressed in the vast majority of SCCs (7).

## Objective

To investigate the expression of tumor suppressor protein p63 in oral squamous cell carcinoma.

## Materials and Methods

**Selection of cases:**
A clinicopathological study was carried out at the Surgical Pathology Center in Davanzo Hospital-Foggia-Italy, between November 2006-Faberuary 2007, involving 93 cases with oral squamous cell carcinoma from formalin – fixed paraffin embedded blocks. Specimens were fixed in 10 % neutral – buffered formalin.

**Tissue processing and staining**
**Immuno histochemical staining**

Serial sections ( 4µm) from formalin – fixed paraffin embedded blocks were cut for each case.

Immuno histochemistry was then performed on the sections that mounted on poly- L- lysine coated glass slides utilizing mouse monoclonal p63 antibody.(3)

after antigen retrieval by pressure cooking with ethylene diamen tetra-acetic acid (EDTA) solution as a buffer solution.

After quenching in 30 % hydrogen peroxide and blocking. The sections were incubated with primary antibody (p63).Then biotinylated anti rabbit immunoglobulin and streptavidin conjugated to horse radish peroxidase were subsequently applied. Finally , 3,3-diaminobenzidine was used for color development and haematoxylin was used for counter staining.

The results of Immuno histochemical staining were evaluated by two observers which appear as brown pigmentation.

## Results

Normal oral mucosa field shows positive expression for P63 by immunohistochemistry in the basal layer area.

### P63 expression in OSCC

P63 expression was observed as positive diffuse reactivity in(38) cases (40.86%) fig. (1), positive basal and Para-basal reactivity in (19) cases (20.43%) fig. (2), positive basal reactivity in (34) cases (36.56%) fig. (3), and negative reactivity in 2 cases (2.15%).

A mean percentage expression and standard deviation (SD) of P63 in OSCC. are shown in table (1).

A statistical correlation between P63 expression and general tumor parameters in OSCC. is shown in table (2).

P63 expression was increased in high grade tumors of size >1.5 cm and metastatic dissemination of lymph nodes with diffuse and basal Para basal pattern of distribution.

Table (3) shows the percentage of expression of P63 in OSCC and the scoring of that expression which shows more than 50% of cases present with score 26-50% of expression.

## Discussion

In this study we have shown that P63 gene a member of P53 family gene is prominently over expressed in about 60.98 % of OSCC. These results were in agreement with Sniezek.J et al. in 2004.

Secondly P63 expression correlated with poorly and moderately differentiated of increased size and was nearly invariably associated with metastatic disease in Lymph nodes.

Experimental studies show that P63 has oncogenic properties in SCCHN and is predominantly involved in maintaining cell survival rather than acting as a directly proliferative factor or as an inhibitor of terminal differentiation, and inhibition of endogenous P63 expression sensitizes cells to the effects of ionizing radiation and chemotherapy, common treatments for SCCHN, so, targeted inhibition of P63 expression in SCCHN could be a useful adjunct for conventional treatments of this disease. (13).

P63 demonstrated as an essential survival factor in HNSCC through its ability to suppress P73 dependent apoptosis (1).

Inhibition of endogenous P63 expression by RNAi induces apoptosis selectively in HNSCC. cells that over express P63 (2).

Knockdown of P63 induces the pro apoptotic bcl-2 family members Puma and Noxa , and both their induction and subsequent cell death are P53 independent but require transactivation of P73 (9).

Inhibition of P73- dependent transcription by P63 involves both direct promoter binding and physical interaction with P73.

In HNSCC cells lacking endogenous P63 expression, bcl-2 is instead up regulated and

can suppress P73- mediated death. Together these findings define a pathway where by P63 promotes survival in squamous epithelial malignancy by repressing a P73 pro apoptotic transcriptional program (2)

Recent work had shown that P63 is essential for survival of SCC cells , raising the possibility that the P63 pathway may be an attractive therapeutic target in SCC. (7).

Experimental analysis found that in normal primary human keratinocytes , the inhibition of endogenous P63 by RNA interference (RNAi) induces P21 expression, inhibits cell cycle progression , and ultimately promotes cellular senescence , in contrast, P63 inhibition in SCC cells induces pro apoptotic bcl-2 family members and rapidly trigger apoptosis, as discussed previously (8).

In conclusion , these data found that the P63 nuclear positivity was extended from the basal-Para basal layers to the whole epithelial layers in both moderately and poorly differentiated tumors , and P63 staining was more uniform and homogenous for the well- differentiated tumor areas. The results of this study are consistent with those from previous analyses of P63 expression in human oral mucosa suggesting that P63 may be associated with the regulation of epithelial differentiation and proliferation in oral squamous cell carcinogenesis (4).

**References**


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**Table(1)** Mean expression of P63 in OSCC**.**

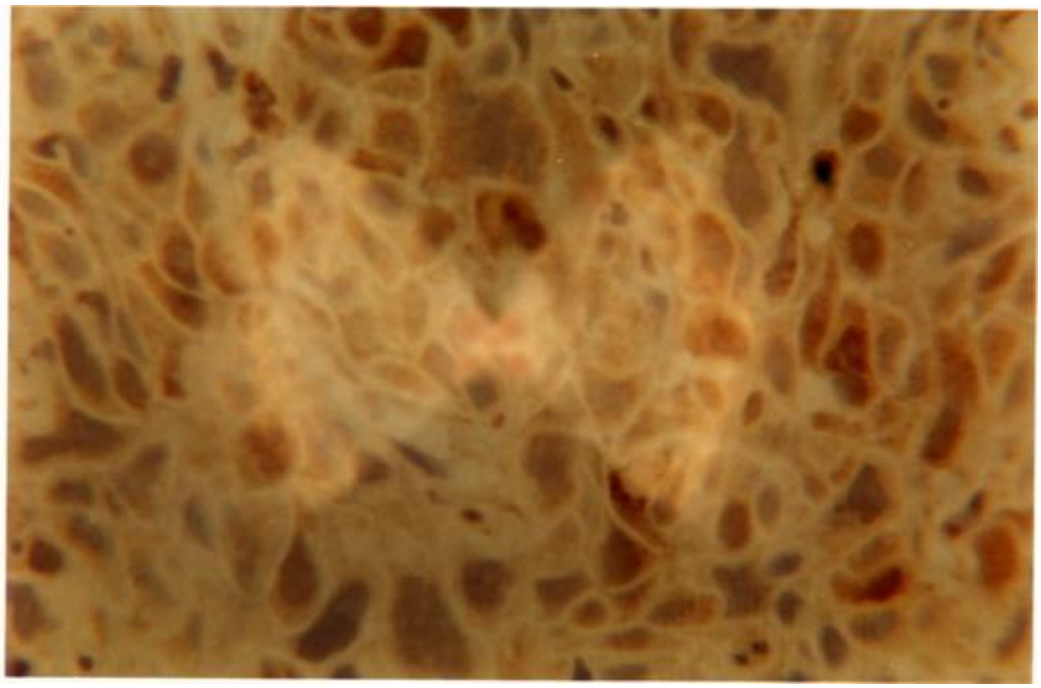
| pattern | Mean | SD |
|---|---|---|
| Basal | 25.31 | ± 9.39 |
| Basal- Para basal | 41.47 | ± 8.42 |
| Diffuse | 44.32 | ± 24.38 |

**Table (2)**Correlation between p63 expression and general tumor parameters in OSCC.

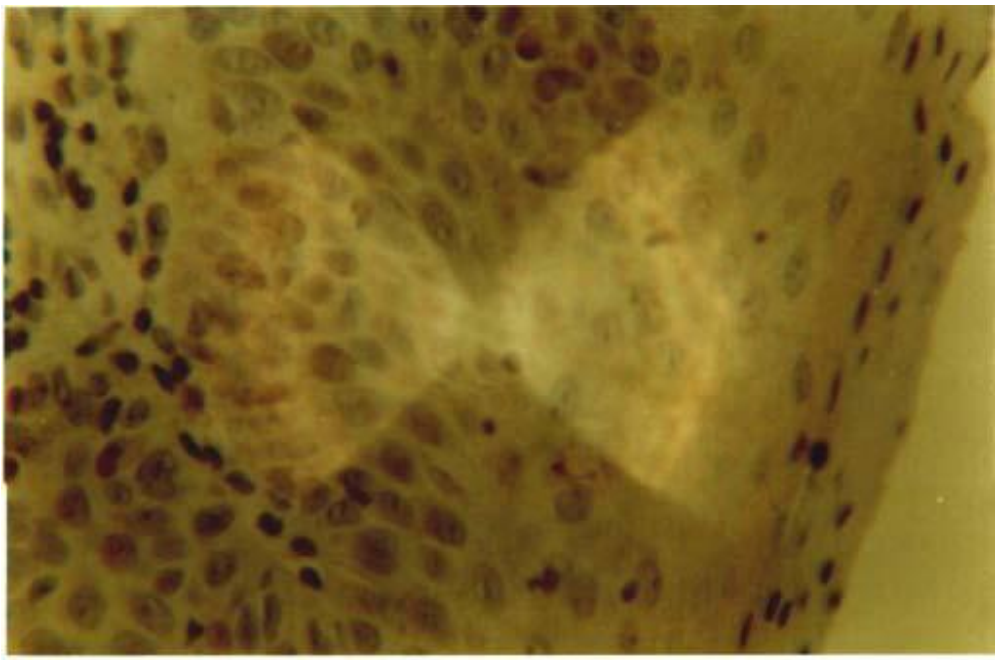
| Variables | No | S. |
|---|---|---|
| Cases<br>**Sex** | 93 | |
| Male | 62 (66.66%) | S |
| Female | 31 (33.33%) | |
| **Grading** | | |
| G1 | 32(34.41%) | G1 vs. G2 N.S |
| G2 | 40 (43.01%) | G1 vs. G3 N.S |
| G3 | 21 (22.58 %) | G2 vs.G3 N.S |
| **Size** | | |
| ≤ 1.5 cm | 32 (35.48 %) | |
| >1.5 cm | 61 (46.52%) | N.S |
| **Lymphnode metastasis** | | |
| negative | 67 (72.04%) | |
| positive | 26 (27.96%) | S |
| **P36 expression** | **No. and percentage** | |
| Basal | 38 (40.86%) | |
| Basal Para basal | 19 (20.43%) | |
| Diffuse | 34 (36.56 %) | |
| negative | 2 (2.15 %) | |

**Table (3)**Scoring and percentage of expression of p63 in OSCC.

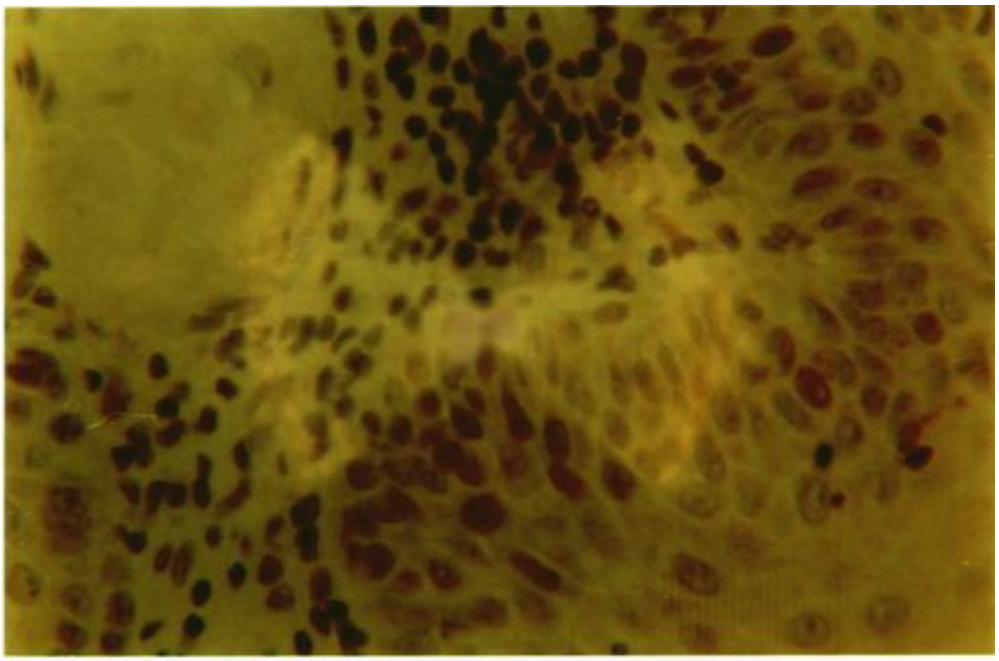
| Expression | No | Score | Percentage |
|---|---|---|---|
| Less than 5% | 2 | 0 | 2.15% |
| 5-25 % | 24 | 1 | 25.8% |
| 26-50% | 51 | 2 | 54.8% |
| 51-75% | 11 | 3 | 11.83% |
| >75% | 5 | 4 | 5.37% |
| Total | 93 | | |



**Figure(1)** Diffuse expression of p63 in OSCC. (100X).



**Figure(2)** Basal - Para basal expression of p63 in OSCC.(100X).



**Figure(3)** Basal expression of p63 in OSCC.(100X).