Original Article

# Detection of Transcription Factor C-MYC in Oral Dysplasia and Squamous Cell Carcinoma by in Situ Hybridization

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**Summary:**

**Background:** Oral squamous cell carcinoma continues to be a major health problem in Iraq as well as in other countries. Many attempts were made to study molecular markers in oral squamous cell carcinoma and to link them to tumor grade, stage and prognosis, besides studying their role in carcinogenesis. The present study has been designed to detect mRNA of c-myc in oral squamous cell carcinoma compared to oral dysplasia and to link the marker to grade and degree of the two pathologies.

**Materials and methods:** Forty two cases, including 30 cases of oral squamous cell carcinoma and 12 cases of oral dysplasia were included in this study. Sections on positively charged slides were made from their paraffin blocks and were used for the detection of c-myc mRNA using in-situ hybridization technique.

**Results:** C-myc mRNA was detected in 9(75%) cases of oral dysplasis and in 24 (80%) cases of oral squamous cell carcinoma. A significant correlation was found between c-myc mRNA score and intensity from one side and the tumor grade from the other side and degree of dysplasia.

**Conclusion:** The results of this study confirm the role of c-myc in oral dysplasia and oral squamous cell carcinoma and the possible transfer from the former to the latter.

**Key Words:** Oral squamous cell carcinoma, oral dysplasia and c-myc.

## Introduction:

Oral malignancy is a major health problem and has a worldwide incidence of 500,000 new cases annually. Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumor of the oral cavity forming about 90% of all tumors and is the seventh most frequent cancer in humans.(1) In Iraq, oral squamous cell carcinoma accounts for about 91.5 % of all oral cancers. Its incidence reaches 4.5 % of all cancer cases according to Iraqi Cancer Registry. (2) Although it is believed that up to a third of oral precancerous lesions may eventually evolve into invasive OSCC over a 10-years interval, no reliable histopathological parameters have been identified that predict their potential for subsequent transformation. Therefore novel molecular predictors of malignant progression are needed to identify oral precancerous lesions at greater risk of invasive transformation as candidates for surgical intervention. (3) An increasing amount of evidence suggests that progression from normal mucosa to cancer is accompanied by morphological and genetic alterations. Genetic abnormalities affect malignant transformation via an imbalance of normal tissue homeostasis involving programmed cell death (apoptosis) in one part in addition to the role of oncogenes and cancer suppressor genes. (4) C-myc oncogene is a transcription factor which is involved in cell proliferation and transformation. An aberrant expression of this gene has been linked to the development and progression of cancer in different body sites. (5, 6, 7) The aim of this study was to detect mRNA c-myc oncogen by in situ hybridization in oral dysplasia and oral squamous cell carcinoma and to correlate between the degree of oral premalignant lesions and the grade of the malignant ones with the marker under study.

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## Materials and Methods:

Thirty cases, histologically diagnosed as oral squamous cell carcinoma including well differentiated carcinoma in 19 cases and poorly differentiated in 11 cases were retrieved from the archives of Oral Pathology Department, College Of Dentistry, University of Baghdad during the period from January 2000 to March 2004. Twelve cases of oral dysplastic premalignant lesions including mild dysplasia in 7 cases, moderate dysplasia in 3 cases and sever dysplasia in 2 cases were also included in the study. Five histologically normal biopsy blocks from patients who underwent extraction of wisdom teeth were used as a control group. Positive control slides for c-myc

from breast cancer cases were also included. Biotinylated Long DNA probes for human c-myc as well as in-situ DNA hybridization/detection system were purchased from Maximbiotech. The kit also contains a biotinylated human house keeping genomic probe as a positive control. Formalin fixed paraffin embedded tissue blocks were used for the preparation of new (4 microns) sections on positively charged slides for the purpose of in-situ hybridization. Working steps included the followings: baking, deparaffinzation, rehydration, washing in buffer, deproteinization, dehydration, hybridization with the specific probe and post hybridization. All were conducted according to the instructions supplied by the kit. Nuclear fast red was used as a counter stain. Using the light microscope, scoring was done at X400 to determine positive hybridization signal. Positive cells were counted in five different high power fields out of 100 nucleated cells. The mean percentage of positive cells was determined assigning cases to one of the four following score categories: (8)

score 0 = < 5 %. score 1 = 5 – 25 %.

score 2 = 25 – 50 %. score 3 > 50 %. The ISH signaling intensity was assessed using a scale of negative, low, moderate and high intensities of signaling. [8] Statistical analysis was conducted using SPSS version 16. Spearman correlation was used to express relative relation between any two ordinal variables.

**Results:**

In-situ hybridization was detected as a blue colorimetric signal in the nucleus or cytoplasm in a red background. Normal oral mucosa showed negative hybridization signals. Positive c-myc mRNA hybridization signal was detected in 9 cases (75%) of oral dysplasia. Four cases (33.33%) were mild dysplasia, 3 cases (25%) were moderate dysplasia and 2 cases (16.67 %) were severe dysplasia. The frequency distribution of positive scores revealed that the highest percentage of cases, 4 (33.33%) were within score 2. Signaling intensity was moderate in 6 cases (50 %). (Table-1) & (Figure-1A & B). A significant positive correlation was found between the degree of oral dysplasia and c-myc positive scores (P<0.05). A significant positive correlation was found between the degree of oral dysplasia and c-myc signaling intensity (P<0.05) as well. (Table-1). Positive c-myc mRNA hybridization signal was detected in 24 cases (80%) of OSCC. Thirteen (43.33%) of these positive cases were well differentiated and eleven cases (36.67%) were poorly differentiated. The frequency distribution of positive scores revealed the highest proportion of cases to be with score 3 which appeared in 10 cases (33.33%) followed by score 2 in 9 cases (30%). Regarding signaling intensity, results revealed high intensity in 12 cases (40%). (Table-2) & (Figure-1C, D & E). Results revealed a highly significant positive correlation between the grade of OSCC and both c-myc positive scores and signaling intensity (P < 0.01). (Table-2). A statistically significant difference was found between well and poorly differentiated OSCC regarding positive c-myc mRNA ISH signals (p<0.05). Table(3). A non-significant statistical difference was found between oral dysplasia and OSCC regarding positive c-myc mRNA ISH signals (p>0.05).Table (4).

**Table (1): Frequency Distribution of C – myc ISH positivity Scores and Signaling Intensity in 12 Cases of Oral Dysplasia.**

| C – myc positivity scores | | | | | |
|---|---|---|---|---|---|
| Oral Dysplasia | 0<br>–<br>No (%) | 1<br>+<br>No (%) | 2<br>++<br>No (%) | 3<br>+++<br>No (%) | Total<br><br>No (%) |
| Mild<br>Moderate<br>Severe | 3(25.00)<br>0<br>0 | 1(8.33)<br>1(8.33)<br>0 | 3(25.00)<br>1 (8.33)<br>0 | 0<br>1 (8.33)<br>2(16.67) | 7(58.33)<br>3(25.00)<br>2(16.67) |
| Total | 3(25.00) | 2(16.67) | 4(33.33) | 3(25.00) | 12 (100) |

**Spearman correlation = 0.694 P 0.012**

| C -myc Intensity | | | | | |
|---|---|---|---|---|---|
| Oral Dysplasia | Negative<br>–<br>No (%) | Weak<br>+<br>No (%) | Moderate<br>++<br>No (%) | Strong<br>+++<br>No (%) | Total<br><br>No (%) |
| Mild<br>Moderate<br>Severe | 3(25.00)<br>0<br>0 | 1(8.33)<br>0<br>0 | 3 (25.00)<br>3 (25.00)<br>0 | 0<br>0<br>2(16.67) | 7(58.33)<br>3(25.00)<br>2(16.67) |
| Total | 3(25.00) | 1(8.33) | 6 (50.00) | 2(16.67) | 12 (100) |

**Spearman correlation = 0.728 P = 0.007**

**Table (2): Frequency Distribution of c-myc ISH positivity Scores and Signaling Intensity in 30 Cases of Oral Squamous Cell Carcinom.**

| C -myc positivity scores | | | | | |
|---|---|---|---|---|---|
| OSCC | 0<br>–<br>No (%) | 1<br>+<br>No (%) | 2<br>++<br>No (%) | 3<br>+++<br>No (%) | Total<br><br>No (%) |
| Well<br>Poor | 6(20.00)<br>0 | 5(16.67)<br>0 | 7(23.33)<br>2 (6.67) | 1 (3.33)<br>9 30.00) | 19(63.33)<br>11(36.67) |
| Total | 6(20.00) | 5(16.67) | 9 (30..) | 10(33.33) | 30 (100) |

**Spearman correlation = 0.756 P < 0.001**

| C -myc Intensity | | | | | |
|---|---|---|---|---|---|
| OSCC | Negative<br>–<br>No (%) | Weak<br>+<br>No (%) | Moderate<br>++<br>No (%) | Strong<br>+++<br>No (%) | Total<br><br>No (%) |
| Well<br>Poor | 6(20.00)<br>0 | 3(10.00)<br>0 | 7 (23.33)<br>2 (6.67) | 3(10.00)<br>9(30.00) | 19(63.33)<br>11(36.67) |
| Total | 6<br>(20.00) | 3<br>(10.00) | 9 (30.00) | 12<br>(40.00) | 30 (100) |

**Spearman correlation = 0.657 P < 0.001**

**Table (3): The difference between well and poorly differentiated OSCC regarding positive ISH signals for c-myc mRNA:**

| OSCC | c-myc | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Well | 13 | 6 | 19 |
| Poor | 11 | 0 | 11 |
| Total | 24 | 6 | 30 |

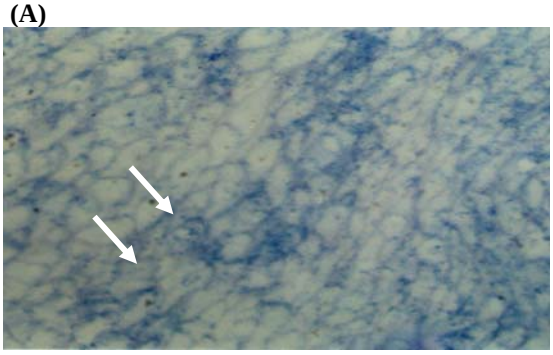
**$X^2 = 4.342$ $P < 0.05$**

**Table (4): The difference between oral dysplasia and OSCC regarding positive ISH signals for c-myc mRNA:**

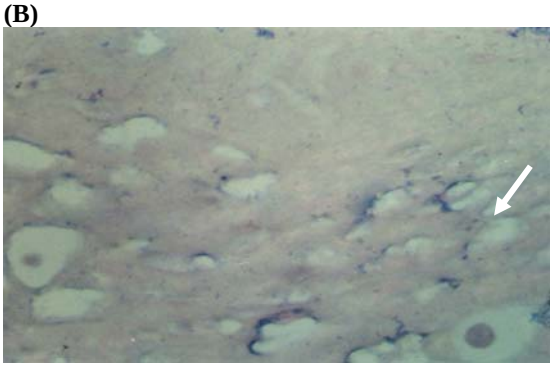
| Pathology | c-myc | | Total |
|---|---|---|---|
| | Positive | Negative- | |
| OSCC | 24 | 6 | 30 |
| Dysplasia | 9 | 3 | 12 |
| Total | 33 | 9 | 42 |

**$X^2 = 0.1272$ $P > 0.05$ NS**

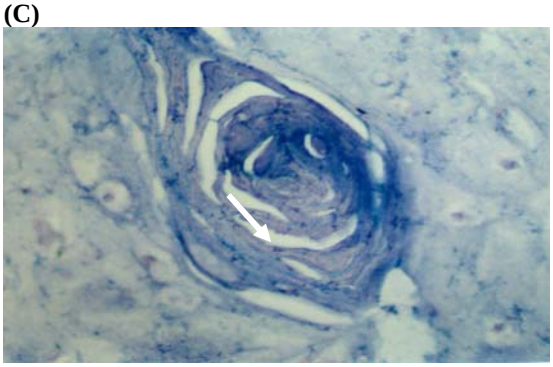
**(A)**



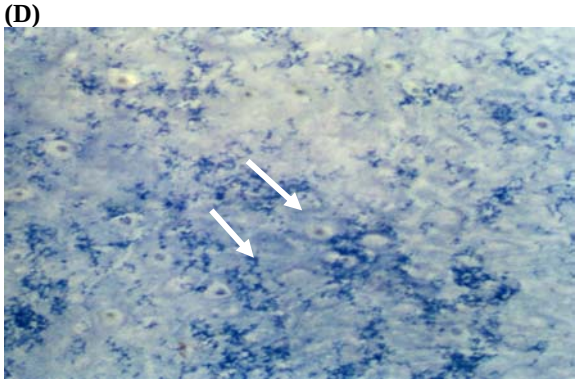
**(B)**



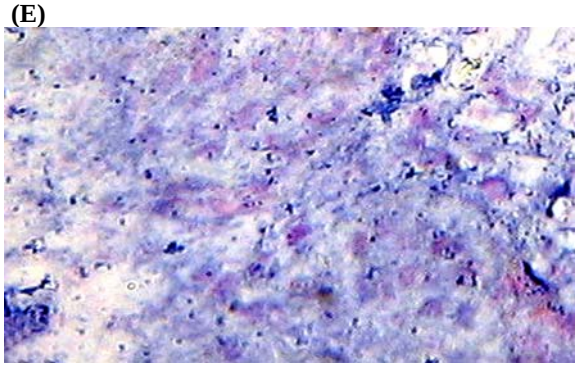
**(C)**



**(D)**



**(E)**



**(F)**

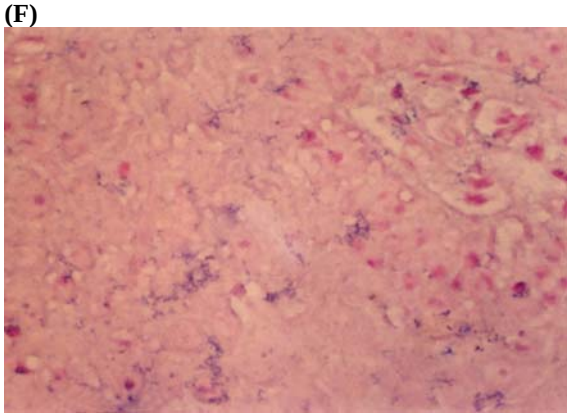


**Figure (1): In situ hybridization for C-myc (bluish-black signals) counter stained with nuclear fast red. A: Oral dysplasia , positive ISH for C-myc,low intensity (X200). B: Oral dysplasia, positive ISH for C-myc, moderate intensity (X400). C: OSCC well differentiated, positive ISH for C-myc mainly in the cell nest, high intensity (X400). .D: OSCC, poorly differentiated, positive ISH for C-myc, high intensity (X200).E: OSCC poorly differentiated , positive ISH for C-myc,high intensity (X400). F: OSCC well differentiated, negative ISH for C-myc (X400). A,C,D&E show high score. B shows low score.**

**Discussion:**

The significant positive correlations which were observed in this study including those between the degree of dysplasia and c-myc mRNA positive scores ($p<0.05$), between the degree of dysplasia and c-myc signaling intensity ($P<0.01$) and between c-myc positive scores and intensity from one side and tumor grade from the other side ($p< 0.01$) reflect the role of c-myc in the progress from dysplasia to carcinoma to different grades of carcinoma. This finding is in agreement with Field 1995 who found over expression of c-myc in oral cancer as a result of gene amplification.(9) The highest frequencies of positive cases being observed in poorly differentiated tumors (100% of poorly differentiated OSCC were c-myc positive) while 68% of well differentiated tumors showed c-myc positively. This finding is in agreement with a previous report that found c-myc over expression by immunohistochemistry to be frequently associated with poorly differentiated oral carcinomas. (10) Mischera & Das found that c-myc expression was absent in normal oral mucosa and became over expressed in higher stages of oral cancer development. (11) In a study applying array-based comparative genomic hybridization to screen OSCC for c-myc gene alteration, high frequency of gene gain was detected. These data could be useful in analyzing pathogenic events involved in the progression of OSCC. (12)

**Conclusion:**

C-myc was found to be an important marker in oral dysplasia and OSCC. Its presence is correlated with the stepwise progression of dysplastic changes and with tumor grade.

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