

P16 Protein and Human Papillomavirus (HPV16, 18) Expressions in Oral Lichen Planus and Squamous Cell Carcinoma

Suhaib Raghieb Muhsin, B.D.S. ⁽¹⁾

Ahlam Hameed Majeed, B.D.S., M.Sc. ⁽²⁾

ABSTRACT

Background: Oral carcinogenesis is a molecular and histological multistage process featuring genetic and phenotypic markers for each stage, which involves enhanced function of several oncogenes and/or the deactivation of tumor suppressor genes, resulting in the loss of cell cycle checkpoints. The progression towards malignancy includes sequential histopathological alterations ranging from hyperplasia through dysplasia to carcinoma in situ and invasive carcinoma. The p16 gene produces p16 protein, which in turn inhibits phosphorylation of retinoblastoma, p16 play a significant role in early carcinogenesis. Human papillomavirus is a well established heterogeneous virus and plays an important role in oral cancers. The aims of the study were to evaluate, compare and correlate the immunohistochemical expression of p16 protein and HPV16/18 with each other in oral lichen planus and oral squamous cell carcinoma, and with various clinicopathological findings.

Materials and methods: Forty formalin-fixed, paraffin embedded tissue blocks (24 cases of oral lichen planus, and 16 cases of oral squamous cell carcinoma) were included in this study, an immunohistochemical staining was performed using anti p16 monoclonal antibody, and anti HPV16/18 monoclonal antibodies.

Results: Positive IHC expression of p16 was found in 11 cases (68.75%) of OSCC, and in 19 cases (79.166%) of OLP. Positive IHC expression of HPV16 was found in 2 cases (12.5%) of OSCC, and in 1 case (4.16%) of OLP. IHC expression of HPV18 showed negative expression in all cases of OSCC, and found only in 1 case (4.16%) of OLP.

Conclusions: This study signifies the statistically non significant correlation between p16 and HPV 16/18 in OLP and OSCC.

Keywords: OLP, OSCC, P16, HPV. (J Bagh Coll Dentistry 2015; 27(4):96-100).

INTRODUCTION

Oncogenesis (carcinogenesis) is the progression from a normal healthy cell to a pre-malignant or a potentially malignant cell - characterised by an ability to proliferate autonomously. Oncogenesis involves a series of genetic steps and also epigenetic-outside the gene-changes. These changes include the aberrant expression and function of molecules regulating cell signalling, growth, survival, motility, angiogenesis (blood vessel proliferation), and cell cycle control ⁽¹⁾. Cancer of the oral cavity is the sixth most common cancer worldwide and account for nearly 3% of all malignancies ⁽²⁾. P16 is a tumor suppressor protein, that in humans is encoded by the CDKN2A gene ^(3,4). It regulates the Rb tumor suppressor pathway by keeping Rb in a hypophosphorylated state, which further promotes the binding of E2F to achieve G1 cell-cycle arrest. The disruption of p16 expression has been reported in various human cancers ⁽⁵⁻⁷⁾.

Human papillomavirus (HPV) is a well-established heterogeneous virus and is important in human carcinogenesis. It not only causes a vast majority of cervical cancers but also plays an important role in anogenital and oral cancers ⁽⁸⁾.

It has been established that HPVs are exclusively epitheliotropic, meaning that their infection is specifically localised in epithelial cells of the host. In order to complete their life cycle, they rely on epithelial differentiation⁽⁹⁾. The present study aimed to evaluate, compare and correlate the immunohistochemical expression of p16 protein and HPV16/18 in oral premalignant lesion and oral squamous cell carcinoma, and with each other in various clinicopathological findings.

MATERIALS AND METHODS

The study samples included 40 formalin-fixed, paraffin embedded tissue blocks (24 OLP, and 16 OSCC) dated from (1975 till 2013), were obtained from the archives of the department of Oral & Maxillofacial Pathology/ College of Dentistry/ University of Baghdad; Al-Shaheed Ghazi Hospital/ Medical City during the period from (1975-2013).

Sections of 4µm thickness were mounted on normal glass slides, stained with H&E for histopathologically re-evaluation. Four other 4µm thick sections 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 p16 and HPV16/18 using Abcam expose mouse and rabbit HRP/DAB

(1) Master student, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.

(2) Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.

immunohistochemical detection kit (Catalog No. ab80436, Cambridge, UK).

RESULTS

Positive p16 Immunostaining was detected as brown nuclear or (nuclear and cytoplasmic) expression.

IHC staining of p16 in OSCC reveals that 5 cases (31.25%) showed negative expression, 5 cases (31.25%) showed weak positive expression, 1 case (6.25%) showed moderate positive expression, and 5 cases (31.25%) showed high positive expression. While in OLP IHC staining of p16 reveals that 19 cases (79.166%) showed negative expression (Cases showing more than 5% of positive cells), and 5 cases (20.833%) showed negative expression (Cases showing less than 5% of positive cells), figures (1,2).

Positive HPV16/18 immunostaining was detected as brown nuclear expression.

Positive IHC expression of HPV16 was found in 2 cases only (12.5%) of OSCC, and 14 cases (87.5%) showed negative expression. HPV16 positivity was found in 1 case only (4.16%) of OLP, and 23 cases (95.83%) showed negative expression. IHC expression of HPV18 showed negative expression in all cases of OSCC. Positive IHC expression of HPV18 was found in 1 case only (4.16%) of OLP, and 23 cases (95.83%) showed negative expression. Figure (3,4,5).

P16 expression were observed in almost cases of OSCC and OLP and according to Chi square test, statistically non significant correlation with clinicopathological findings (age, sex, tumor site, tumor grade) except tumor site of OSCC in p16 was statistically significant, while HPV16/18 expression was detectable in few cases of OSCC and OLP and correlation between the expression of markers (P16, HPV16, HPV18) were non significant statistically in OSCC and OLP ($P=0.757, 0.327, 0.874$) respectively, as clarified in tables (1,2,3).

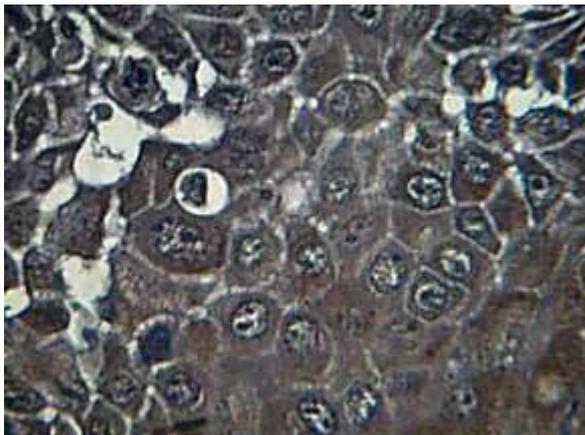


Figure (1): Positive Expression of p16 in Well Differentiated OSCC (40X).

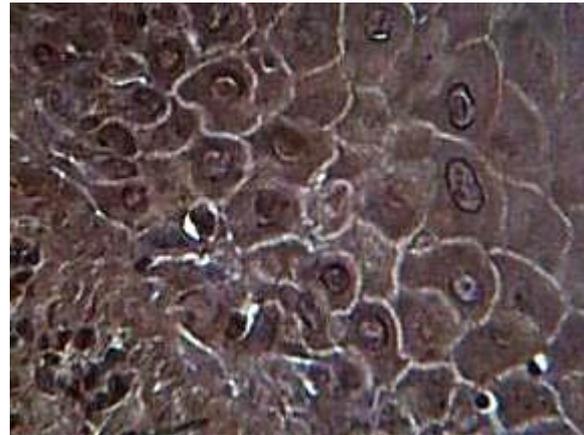


Figure (2): Positive Expression of p16 in OLP (40X).

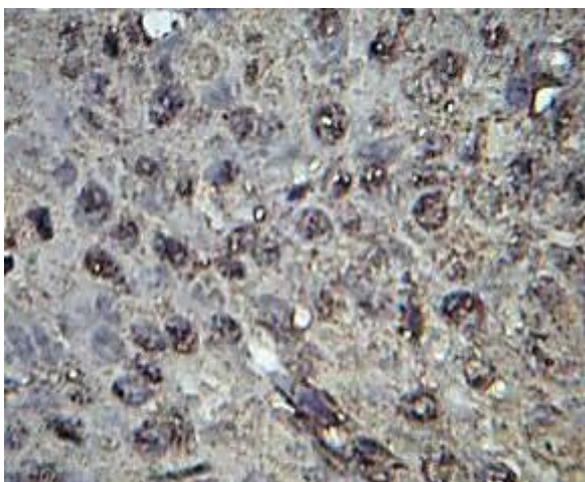


Figure (3): Positive Expression of HPV16 in OSCC (40X).

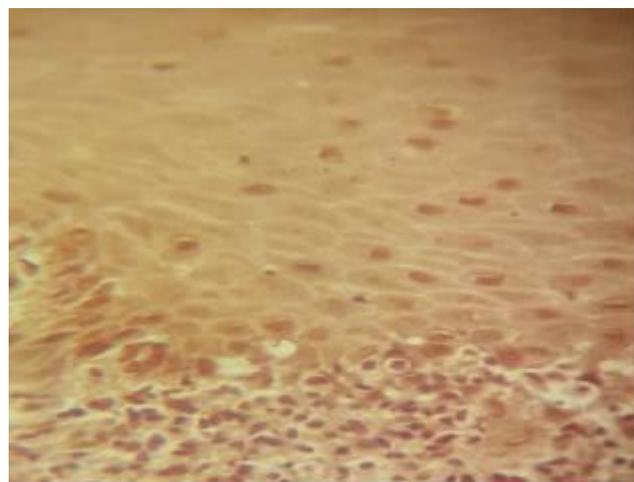


Figure (4): Positive Expression of HPV16 in OLP (40X).

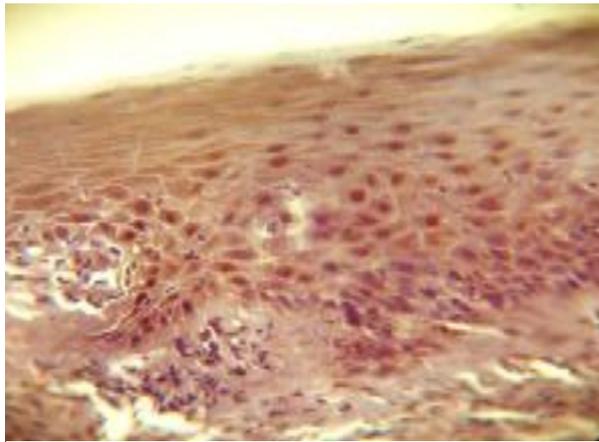


Figure (5): Positive Expression of HPV18 in OLP (40X).

Table (1): Correlation between OSCC and OLP of P16 Expression

| P16 | +Ve | -VE | N | X ² | Sig |
|-------|-----|-----|----|----------------|-------|
| OLP | 19 | 5 | 24 | 0.096 | 0.757 |
| OSCC | 12 | 4 | 16 | | |
| Total | 31 | 9 | 40 | | |

Table (2): Correlation between OSCC and OLP of HPV16

| HPV16 | +Ve | -VE | N | X ² | Sig |
|-------|-----|-----|----|----------------|-------|
| OLP | 1 | 23 | 24 | 0.096 | 0.327 |
| OSCC | 2 | 14 | 16 | | |
| Total | 3 | 37 | 40 | | |

Table (3): Correlation between OSCC and OLP of HPV18

| HPV16 | +Ve | -VE | N | X ² | Sig |
|-------|-----|-----|----|----------------|-------|
| OLP | 1 | 23 | 24 | 0.684 | 0.871 |
| OSCC | 0 | 16 | 16 | | |
| Total | 1 | 39 | 40 | | |

DISCUSSION

The present study is not a large epidemiological one that expressed the incidence and prevalence of different clinicopathological features of OLP and OSCC, however, there was a close correlation between the present data and other published data concerning the incidence of OLP and OSCC in Iraq in the past studies records and studies in other parts in the world^(10,11).

Assessment of p16 Immunohistochemistry:

P16 positivity was found in (68.75%) of OSCC cases. Concerning the correlation between clinicopathological findings of OSCC cases and p16, the present study showed statistically significant correlation between p16 expression and the tumor site, while there was non significant difference between p16 with age, sex, site, and grades of OSCC. The discrepancies in the result of p16 expression in this result and with other studies^(12,13) could be attributed to limited sample size of the current study.

Concerning OLP cases the results of this study showed that positive expression of p16 was observed in 19 cases (79.166%) and negative expression in 5 cases (20.833%). Montebugnoli et al and Poomsawat et al detected p16 in 64% of OLP samples and reported that p16 expression in 65.2% of OLP cases respectively^(14,15). Different cancer-causing agents may lead to p16INK4a gene inactivation as well as altered p53 and pRb tumor suppressive pathways^(16,17). The loss of p16 expression as a result of promoter

hypermethylation is an early event in oral carcinoma and a useful biomarker for predicting local recurrence in carcinoma of the tongue⁽¹⁸⁾. However, the role of p16 hypermethylation as a predictive risk factor for OSCC or disease recurrence remains unclear and contradictory⁽¹⁹⁾.

Assessment of HPV Immunohistochemistry:

In OSCC, The present study showed negative HPV16 immunoreactivity in 14 cases (87.5%) and only 2 cases (12.5%) showed positive expression whereas in HPV18, all cases showed negative expression. This finding was in agreement with studies in other part of the world⁽²⁰⁻²²⁾.

In current study, most of OSCC cases that showed negative expression found in cytoplasm. Concerning the correlation between clinicopathological findings of OSCC cases and HPV16 and 18, the present study showed non significant statistical correlation between HPV16, 18 expression with age, sex, site and grade were found⁽²³⁻²⁸⁾.

Regarding OLP, the result of this study showed positive expression of HPV16 only in 1 case and other 23 cases (95.83%) showed negative expression and same result was found in HPV18.

Few studies employed immunohistochemical detection of HPV 16 in oral lichen planus, in a Turkish study by Yildirim et al 21% of the cases were positive by immunohistochemistry⁽²⁹⁾. Concerning correlation between p16 and HPV16/18 in oral squamous cell carcinoma and

oral lichen planus, the present study showed statistically non significant correlation between p16 and HPV16,18 in OSCC and OLP.

Nemes et, al., showed that over-expression of p16INK4a proteins in OSCC did not correlate with HR-HPV types⁽³⁰⁾. In addition, very high p16 expression observed not only in HPV positive groups but also in other groups in the absence of HPVs. Therefore, it revealed unconvincing support for previous claims on the HPV-p16 relationship⁽³¹⁻³⁴⁾.

Concerning OLP, non significant correlation was observed in p16 and HPV16 and 18. Montebugnoli et al found p16INK4 expression was detected in 26 specimens, while HPV was found in four lesions: three low-risk HPV, and one high-risk HPV. All HPV-positive lesions also showed p16INK4A overexpression, whereas 22 cases of overexpressed p16INK4A were HPV-negative⁽³⁵⁾.

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