Immunohistochemical expression of P16 and HER2/neu in normal oral mucosa, oral epithelial dysplasia, and oral squamous cell carcinoma

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ABSTRACT

Background: Oncogenesis in the oral cavity is widely believed to result from cumulative genetic alterations that cause a transformation of the mucosa from normal to dysplastic to invasive carcinoma. The p16 gene produces p16 protein, which in turn inhibits phosphorylation of retinoblastoma (Rb), p16 play a significant role in early carcinogenesis. A number of epidermal growth factor receptor (EGFR) family, HER2/neu, has received much attention because of its therapeutic implications. The aims of the study were to evaluate and compare the immunohistochemical expression of the cell cycle protein P16 INK4a and c-erbB2 (HER2/neu) in NOM, OED, and OSCC. Correlate both marker expression with each other as well as with various clinicopathological findings.

Materials and methods: Sixty two formalin-fixed, paraffin embedded tissue blocks (20 cases of normal oral mucosa, 17 cases of oral epithelial dysplasia, and 25 cases of oral squamous cell carcinoma) were included in this study, an immunohistochemical staining was performed using anti p16 monoclonal antibody, and anti HER2/neu polyclonal antibody.

Results: Positive IHC expression of p16 was found in 18 cases (90%) of NOM, 16 cases (94.1%) of OED and in 20 cases (80%) of OSCC. Positive IHC expression of HER2/neu was almost undetectable in NOM, while it was found in 9 cases (52.9%) of OED, and in 15 cases (60%) of OSCC. The correlation between the expression of both markers were statistically highly significant in NOM, significant in OED, and non significant in OSCC.

Conclusions: This study signify the important role of p16 and HER2/neu in oral carcinogenesis and in the evolution of the mucosa from normal to dysplastic to invasive carcinoma

Keywords: NOM, OED, OSCC, P16, HER2/neu. (J Bagh Coll Dentistry 2014; 26(2): 94-98).

الخلاصه

الخلفيه: عملية التسرطن في التجويف الفمي غالبا ما تحدث نتيجة تراكم التغييرات الجينيه التي تحول الغشاء المخاطي الفموي و بخطوات عديده من الطبيعي الى الحثل النموي الطلائي ثم النسيج السرطاني المتغلغل.المورث الجيني p16 فهو ينتج بروتين P16 الذي في المقابل يمنع فسفرة بروتين Rb, وكذلك فان بروتين p16 يلعب دورا مهما في عملية التسرطن المبكر. أما HER2/neu فهو أحد افراد عائلة مستقبلات عامل نمو خلايا الأدمه (EGFR) والذي حظي اهتماما كبيرا بسبب تطبيقاته العلاجيه.

اهداف الدراسه: تقييم التعبير المناعي النسيجي الكيميائي لبروتين p16 INK4a ومحفز HER2/neu في النسيج المخاطي الفموي الطبيعي ,و الحثل النموي الطلائي الفموي ,وسرطان الفم الحرشفي . وكذلك اكتشاف علاقة المورثات الجينه p16 و HER2/neu مع بعضها وكذلك علاقتهما مع المقاييس السريريه والمرضيه

المواد وطرق العمل :تشمل الدراسه اثنان وستون حاله مختاره من قوالب شمع البارافين, تضمنت 20 حاله من الغشاء الفموي الطبيعي و 17 حاله من الحثل النموي الطلائي الفموي و25 حاله من سرطان الفم الحرشفي.و اجراء الفحوصات المناعيه الكيميائيه النسيجيه باستخدام المؤشرات p16 وp16 HER2/neu.

النتائج : اظهرت الدراسه الحاليه ان التعبير الايجابي المناعي النسيجي الكيميائي لبروتين p16 كان قد تبين في 18 حاله (90%) من الغشاء الفموي الطبيعي و الذي اظهر تعبير ايجابي ضعيف, كما وظهر التعبير الايجابي في 16 حاله (94.1%) من الحثل النموي الطلائي الفموي ., بينما كان التعبير الايجابي لبروتين p16 في 20 حاله (80%) من سرطان الفم الحرشفي. التعبير الايجابي لمحفز HER2 لم يتم الكشف عنه في الغشاء الفموي الطبيعي, بينما في الحثل النموي الطلائي الفموي كان الظهور الايجابي ل محفز HER2 قد تبين في تسع حالات(52.9%), بينما التعبير الايجابي لمحفز HER2 كان قد وجد في 15 حاله (60%) من سرطان الفم الحرشفي. العلاقة الاحصائيه بين المؤشرين p16 وHER2/neu كانت معنويه للغايه في الغشاء الفموي الطبيعي , وكذلك كانت العلاقه معنويه في الحثل النموي الطلائي الفموي , في حين انه لم تكن هناك علاقه معنويه بين المؤشرين في سرطان الفم الحرشفي.

الاستنتاجات :كشفت هذه الدراسه على الدور المهم لبروتين p16ومحفز HER2/neu في عملية التسرطن وكذلك في تطور الغشاء المخاطي الفموي من الطبيعي الى الحثل النموي ثم الى سرطان الفم الحرشفي.

INTRODUCTION

Oral carcinogenesis is a multi-step process involving gene mutations and chromosomal abnormalities [(1)]. The transition from normal oral epithelium to oral dysplasia and cancer results from accumulated genetic and epigenetic alterations [(2)].

The best-known precursor lesion is epithelial dysplasia, which is histologically detectable and often presents clinically as white or red mucosal patches called leukoplakia and erythroplakia [(3)].

Oral cancers account for nearly 3% of all malignancies, and they are the sixth cancer by incidence worldwide, with epidemiologic variations existing between different geographic regions [(4-6)].

The cell cycle protein (p16) is a well-known tumor suppressor protein, composed of 156 amino acids encoded by a three exons of the p16 gene. 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. [(7-9)].

The transmembrane tyrosine kinase receptor constitute the ErbB receptor family and comprised of four different receptors known as ErbB1 (also referred to as (EGFR), ErbB (HER2/neu in rodents), ErbB3 (HER3), and ErbB4 (HER4) [(10-]

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[12]. C-erbB-2proto-oncogene (HER/NEU/neu) encodes a 185 transmembrane protein product of tyrosine kinase family, with an extensive homology to the epidermal growth factor receptor [13] and can be activated by hetero oligomerization with the other members of the ErbB family [14].

The lack of a unique marker of OSCC has long been a problem in the early detection of OSCC. It would be necessary to discover more reliable and efficient markers to characterize the malignant transformation of oral epithelium [15, 16].

This study aimed to evaluate and compare the expression of P16 and HER2/neu in normal oral mucosa, oral epithelial dysplasia, and oral squamous cell carcinoma, and to correlate both marker expression with each other, as well as with various clinicopathological findings including (age, sex, clinical presentation, tumor site, tumor grade).

MATERIALS AND METHODS

The study samples included sixty two formalin-fixed, paraffin embedded tissue blocks (20 NOM, 17 OED, and 25 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 / Baghdad; and Al Kadhimiya teaching Hospital. Sections of 4μm thickness were mounted on normal glass slides, stained with H&E and histopathologically re-evaluated. 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 using Abcam expose mouse and rabbit HRP/DAB immunohistochemical detection kit (Catalog No. ab54210, Cambridge, UK). And polyclonal antibody HER2/neu using Rabbit Anti-Human c-erbB-2 Oncoprotein (Catalog No. A 0485) Dako Denmark immunohistochemical detection kit was used.

RESULTS

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

IHC staining of p16 in NOM reveals that 2 cases (10%) showed negative expression, 18 (90%) cases showed weak positive expression. And in OED, 1 case (5.9%) showed negative expression, 1 case (5.9%) showed weak positive expression, 6 cases (35.3%) showed moderate positive expression, and 9 cases (52.9%) showed high positive expression. While in OSCC, IHC staining of p16 reveals that 5 cases (20%)

showed negative expression, 3 cases (12%) showed weak positive expression, 2 cases (8%) showed moderate positive expression, and 15 cases (60%) showed high positive expression. fig (1,2,3)

Positive HER2/neu immunostaining was detected as brown membranous or (membranous and cytoplasmic) expression. Regarding HER2/neu expression in NOM, all cases (100%) showed negative expression. And in OED, 8 cases (47.1%) showed negative expression, 5 cases (29.4%) showed weak positive expression, and 4 cases (23.5%) showed strong positive expression. While in OSCC HER2/neu immunostaining reveals that 10 cases (40%) showed negative expression, 10 cases (40%) showed weak positive expression, and 5 cases (20%) showed strong positive expression. Fig (4,5,6).

Regarding the correlation between p16 and HER/neu expression in each group and according to Mann-Whitney U test, the results revealed a statistically highly significant correlation in NOM (p-value= 0.000), and significant correlation in OED (p=0.02), while the results revealed non significant correlation in OSCC (p=0.14). As clarified in table (1). Regarding groups' comparison in each marker, the results revealed statistically highly significant correlation (p=0.000). As clarified in table (2).
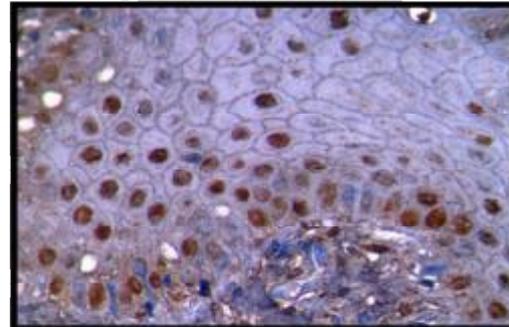

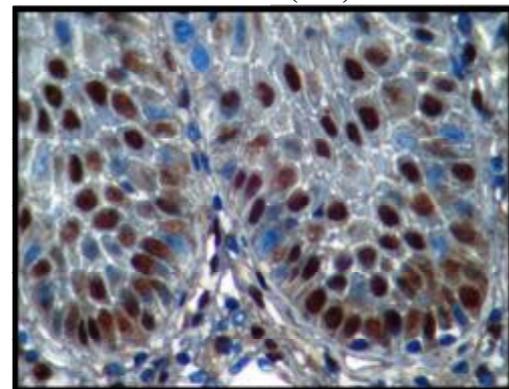**Figure 1: Positive nuclear expression of p16 in NOM (40x).**

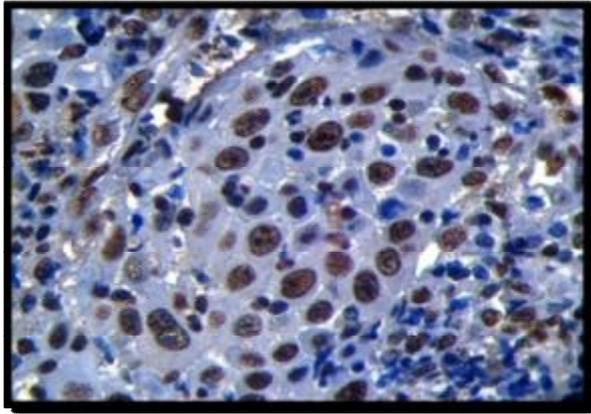**Figure 2: Positive nuclear & cytoplasmic expression of p16 in severe dysplasia (40x).**

Figure 3: positive nuclear expression of p16 in moderately differentiated SCC,(40x).
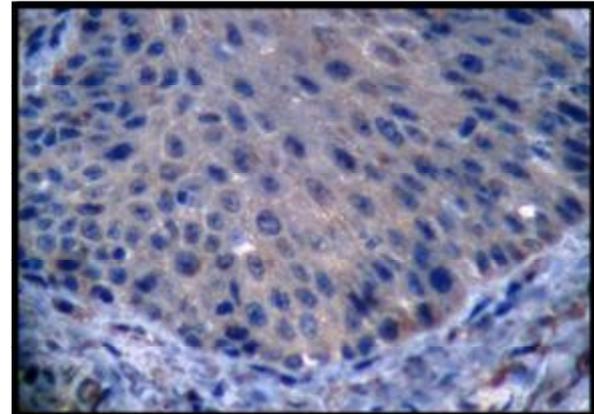

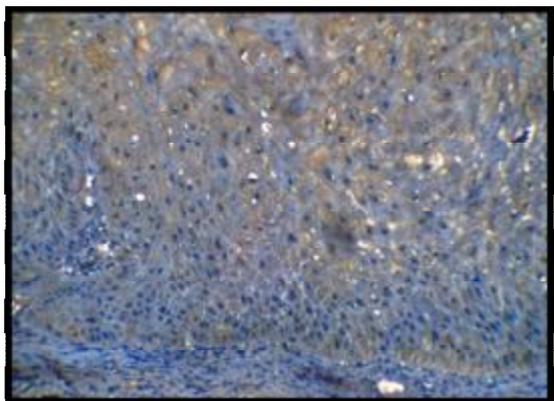**Figure 4: Negative HER2/neu expression in NOM (tongue) (40x)**

Figure 5: Positive membranous & cytoplasmic expression of HER2/neu in severe dysplsia (10x).
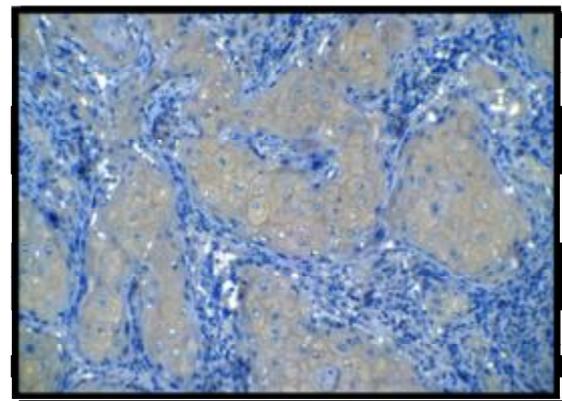

Figure 6: Positive membranous & cytoplasmic expression of HER2/neu in moderately differentiated SCC (10x)

Table 1: Descriptive statistics and markers' comparison in each group

Groups	Markers	Descriptive Statistics				Comparison	
		N	Mean	S.D.	S.E.	Mann-Whitney U test	p-value
NOM	P16	20	12.05	5.86	1.31	-3.93	0.000 (HS)
	HER2	20	4.65	2.98	0.67		
ED	P16	17	62.24	32.98	8.00	-2.36	0.02 (S)
	HER2	17	32.24	30.93	7.50		
SCC	P16	25	52.20	36.16	7.23	-1.47	0.14 (NS)
	HER2	25	31.88	29.71	5.94		

Table 2: Descriptive statistics and groups' comparison in each marker

Markers	Groups	Descriptive Statistics				Comparison	
		N	Mean	S.D.	S.E.	Kruskal-Wallis test	p-value
P16	NOM	20	12.05	5.86	1.31	20.66	0.000 (HS)
	ED	17	62.24	32.98	8.00		
	SCC	25	52.20	36.16	7.23		
HER2/neu	NOM	20	4.65	2.98	0.67	18.29	0.000 (HS)
	ED	17	32.24	30.93	7.50		
	SCC	25	31.88	29.71	5.94		

DISCUSSION

This study is not a large epidemiological one that expressed the incidence and prevalence of different clinicopathological features of OED and OSCC, however, there was a close correlation between the present data and other published data concerning the incidence of OED and OSCC in

Iraq in the past studies records and studies in other parts in the world [17,18].

Assessment of p16 immunohistochemistry

The results of the present study showed that positive immunostaining of p16 was found in 90% of normal oral mucosa cases with variable nuclear and cytoplasmic expression. This result agrees with Tarakji et al. [19].

As p16 is involved in cell cycle regulation its expression may vary with cell turnover times in the oral mucosa which have been shown to be variable in different types of oral mucosa [20]. The activation of p16 expression can be triggered by DNA damage, oncogenic stress or physiological aging [21].

Concerning OED cases the results of this study showed that positive expression of p16 was observed in (94.1%) of OED cases. Regarding correlation between p16 and clinicopathological features, there was statistically non significant correlation, which agreed with Bradley et al. [22].

P16 positivity was found in (80%) 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 [23,24]. These differences due to limited sample size of this study.

Different cancer-causing agents may lead to p16^{INK4a} gene inactivation as well as altered p53 and pRb tumor suppressive pathways [25,26]. These changes may result in either loss or overexpression of p16^{INK4a} in oral dysplasia and OSCC.

HPV oncogenes are frequently found in oropharyngeal squamous cell carcinomas that display concomitant increased p16^{INK4a} expression [27,28]. Other relevant etiopathological agents that may influence p16^{INK4a} expression are smoking and smoke-less tobacco use [19,27]. The oral mucosa of smokers, express p16^{INK4a} more frequently when compared to individuals that do not use tobacco, and this could be attributed to the component of tobacco smoke (nicotine), which is well known to significantly stimulate cell growth, epithelial cell DNA synthesis and cell proliferation that stimulated at nicotine concentrations lower than those obtained in blood after smoking [27,29].

Assessment of HER2 / neuimmuno-histochemistry

The present study showed negative HER2/neu immunoreactivty in normal oral mucosa. And in OED it was found in (52.9%). These results were in agreement with Jubair [18] that showed HER2/neu expression in NOM was almost undetectable. According to clinicopathological correlation of HER2/neu and OED, the results of this study showed statistically non-significant correlation, which agrees with Jubair [18]. HER2/neu positivity was found in (60%) of OSCC cases. Agree with [18] that showed higher HER2/neu expression in the OSCC group.

The overexpression of HER2/neu could be a potential useful marker in distinguishing non-cancer and cancer, as shown in this study. Once the overexpression of HER2/neu is found in cases with benign or precancerous lesions in the oral cavity, care should be taken in the follow-up of such patients. Early treatment with excision of the ED showing expression of HER2/neu may be required [30,31]. Activation of EGFR family by a variety of ligands is necessary for normal growth and differentiation [32].

The present study showed statistically highly significant correlation between p16 and HER2/neu in NOM, and significant correlation between them in OED, and showed statistically non significant correlation between them in OSCC.

Correlation between p16 and HER2/neu in each group

This is the first study in Iraq and other parts in the world assessing the correlation between p16 and HER2/neu immunohistochemical expression in NOM, OED, and OSCC. Since this is a pioneer research in assessing that correlation, so the comparison could be withdrawn from other studies using another technique ,which is agree with [33] that showed there was non-significant correlation between p16 deletion and HER2/neu amplification in oral squamous cell carcinoma by using fluorescent in situ hybridization.

The present study showed highly significant correlation in each marker regarding groups' comparison. This means that p16 and HER2/neu play a role in oral carcinogenesis.

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