Immunohistochemical expression of HOXA1, and Ki-67 proteins of oral squamous cell carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most prevalent malignant neoplasm of the oral cavity and constitutes a major health problem in developing. In the last 30 years, the 5-year survival rate of patients with oral SCC has not improved despite advance in diagnostic techniques. To improve early diagnosis for this deadly disease, new biological markers are needed. *HOX* genes encode homeodomain-containing transcription factors involved in the regulation of cellular proliferation and differentiation during embryogenesis. *HOX* gene expression has been described in several adult tissues, where they performed important roles in maintaining homeostasis. Few studies have suggested that *HOXA1* plays a role in tumorigenesis. Besides being overexpressed in several tumors, *HOXA1* influences numerous cellular processes including proliferation, apoptosis and epithelialmesenchymal transition (EMT), and *HOXA1* overexpression is sufficient for malignant transformation ofnontumorigenic epithelial cells. *Ki-67* is a specific marker of proliferation and the expression of which is strictly associated with cell proliferation and is widely used in pathology as a proliferation marker to measure the growth fraction of cells in human tumors.The aims of this study were to evaluate the immunohistochemical expression of HOXA1 & Ki-67 in OSCC & to correlate the expression of the studied markers with the clinicopathological findings and with each other

Materials and Methods: Thirty formalin-fixed, paraffin- embedded tissue blocks of oral squamous cell carcinoma were included in this study. H&E stain was done for each block for reassessment of histological examination. An immunohistochimical stain was performed using anti *HOXA1* and anti *Ki-67* poly clonal antibodies.

Results:The expression of *HOXA1* and *Ki-67*were positive in all oral squamous cell carcinoma cases & in all layers (100%), while the expression was restricted to the basal and supra basal layer in normal oral mucosa. Statistically non-significant correlation observed between each marker with clinico-pathological parameters. While a statistically significant association was found between the expressions of two markers, (p-value= 0.027).

Conclusion: The statistically significant association observed between expressions of *HOXA1* with the specific marker of proliferation *Ki-67*. This suggested important role in oral SCC development and progression.

Keywords: OSCC, HOXA1, Ki-67. (J Bagh Coll Dentistry 2014; 26(2): 74-78).

الخلاصة

الخلفية:

سرطان الخلايا الحرشفية هو السرطان السائد في التجويف الفمي ويمثل المشكلة الرئيسية المؤدية للوفاة في بلدان العالم الثالث. وبالرغم من تطور التقنيات الطبية التشخيصية إلا إن مستوى سنوات البقاء الخمسة المعتمدة في علم الأورام لم يتطور بشكل مفيد في السنوات الثلاثين الاخيرة. إن الكشف المبكر لسرطان الخلايا الحرشفية الفموي مهم جدا للحد من خطورته ولذلك تم التركيز على إيجاد واسمات بيولوجية جديدة ومنها جين HOXA1 وهو احد افراد عائلة جين HOX التابعة لجينات Homeobox المحفزة للنمو والتمايز اثناء النمو الجنيني والتكوين العضوي وقد يظهر في الأنسجة البالغة عند الحاجة ايضا. وقد اثبتت دراسات حديثة دوره المسرطنالفعال في العديد من الامراض السرطانية حيث يظهر بشكل غير متوازن في الأنسجة . Ki-67 هو مؤشر التكاثر الرئيسي في النواة وهو المساعد في كشف وجود اي انقسامات في الأنسجة وبالتالي فله فائدة عظيمة في التنبؤ والكشف المبكر لسرطان الفم. تهدف الدراسة الحالية الى التحري والتحقق من ظهور جين HOXA1 في السرطان الحرشفي للفم وربط ظهور الجين بمؤشر التكاثر Ki-67 .وكذلك ربط ظهور كل منهما مع المعطيات السريرية المرضية لسرطان الفم الحرشفي.

المواد والطرق: تضمنت هذه الدراسة ثلاثين عينة استرجاعية لأشخاص مصابين بسرطان الفم الحرشفي والتي استخرجت من المقاطع النسيجية المثبتة بالفورمالين والمطمورة بشمع البارافين وجرى صبغ كل عينة بصبغتي الهيماتوكسلين والايوسين لإعادة تقييمها لغرض الفحص النسيجي المرضي. بعد لك اجريت الصبغات الكيميائية النسيجية المناعية باستخدام مضاد HOXA1 ومضاد Ki-67 على شرائح نسيجية دقيقة من العينات.

النتائج: أظهرت الدراسة أن اكثر حالات هذا السرطان تقع في الاعمار التي تفوق الخمسين عاماً وأن معظم تلك الحالات تركزت في الذكور وبنسبة (70 %) .اما نسبة إصابة الذكور الى الاناث فقد اظهرت هذه الدراسة إلى انها تساوي:(1:2). كذلك اظهرت الدراسة ان معظم الحالات كانت في اللسان (36,7%) ومعظمها ظهرت سريريا بشكل اورا م (73,3%) .اما الفحوصات النسيجية المرضية لهذه الدراسة فقد أظهرت ان (43,3 %) من الحالات السرطانية هي من النوع المتوسط التمايز و(40,0%) من النوع الواضح التمايز لسرطان الفم الحرشفي . اظهرت هذه الدراسة ايضاً ان تعبير HOXA1 ومؤشر التكاثر Ki-67 كان ايجابياً في الطبقة السفلى فقط من النسيج المخاطي الفموي الطبيعي، بينما كان ايجابياً في كل طبقات النسيج الحرشفي لسرطان الفم. كذلك اظهرت هذه الدراسة وجود علاقة واضحة بين ظهور جين HOXA1 ومؤشر التكاثر Ki-67 مما يدل على الدور المهم لجين HOXA1 كعامل مسرطن وذلك بواسطة تحفيز انقسام الخلايا وبالتالي النمو السرطاني الفموي واخيرا بينت هذه الدراسة عدم وجود اية علاقة بين العاملين السابقين والمتغيرات السريرية المرضية الاخرى المصاحبة لسرطان الفم الحرشفي.

الاستنتاجات: تلازم وجود جين HOXA1 مع مؤشر التكاثر Ki-67 دلالة على دور جين HOXA1 في انقسام الخلايا والنمو السرطاني لذلك تقترح هذه الدراسة اجراء دراسات جديدة بعينات اكثر عددا لمعرفه الدور الحقيقي لجين HOXA1 او عضو اخر لجين HOX مع مؤشر التكاثر Ki-67 او مع مؤشر تكاثر اخر.

INTRODUCTION

Squamous cell carcinoma (SCC) accounts to more than 90% of malignant tumors of the oral cavity and oropharynx. It is often related to considerable mortality and morbidity rates, and presents a variable etiology related to alcohol and tobacco abuse associated with genetic factors [1,2].

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The homeobox genes, is a master regulators of morphogenesis and cell differentiation during embryogenesis, have emerged as potential candidates to be also involved with carcinogenesis. This important family of genes codes regulatory proteins that act as transcriptional factors controlling the development of several tissues including orofacial tissue [3,4]. HOXA1 is a member of HOX genes family which issubgroup of homeobox genes have important role in OSCC development and

progression. Ki-67 is a cell cycle associated human nuclear protein present in peri-chromosomal region [5].

The estimated half-life of Ki-67antigen is 60-90 minutes, and the Ki-67antigen starts to be expressed in S phase, progressively increasing through S and G2 phase and reaching a plateau at mitosis. After cell division, the cell return to G1with a stock of Ki-67antigen, whose level decreases rapidly during this phase [6,7].

This study aimed to:

- Evaluate the immunohistochemical expression of *HOXA1* and *Ki-67* markers in oral squamous cell carcinoma.
- Correlate the expression of either marker with each other and with the Clinico-pathological parameters (age, sex, tumor site, clinical presentation, and histopathological grades) of OSCC.

MATERIALS AND METHODS

A retrospective study was performed on thirty-formalin- fixed paraffin embedded blocks of OSCCwere collected from the archives of Oral Pathology laboratory, College of Dentistry, Baghdad University, Al-Kadhimiya teaching hospital, and Al-Shaheed Ghazi Hospital/ Medical City / Baghdad from (2010-2013).The diagnosis of each case was confirmed by examining the Hematoxylin and Eosin (H&E) sections by two experienced pathologists. Four micrometer thick sections were cut and mounted on positively charged slides and stained immunohistochemically with monoclonal antibodies using anti HOXA1 and anti Ki-67polyclonal antibodies (Abcam UK). Abcam expose mouse and rabbit HRP/DAB immunohistochemical detection kit (Catalog No. ab80436, Cambridge, UK) was used.

RESULTS

Clinicopathological Findings of OSCC cases were designed as follows: Most of the cases 21 (70%) aged were above 50 years and the majority of the cases were males 21 (70 %). The most common site was the tongue 11 cases (36.7%) and most of the cases were presented as mass22 cases (73.3%).

Histopathological examination showed that 13 cases of OSCC (43.3%) were moderately differentiated, followed by 12 cases (40%) well differentiated and 5 cases (16.7%) were poorly differentiatedas shown in table (1). Immunohistochemical staining with *HOXA1*primary antibody showed that *HOXA1* expression was positive in all examined OSCC specimens andwas observed as a nuclear stain restricted to the basal and suprabasal layers in healthy mucosae figures (1), whereas a broad positivity with variable distribution and intensity was found in the OSCC samples as shown in figures (2,3). score +1 was found in 26.6% (8 cases), score +2 and score +3 both found in 36.7% (11 cases) table(2) . Immunohistochemical staining with *Ki-67* primary antibody showed that *Ki-67* expression was positive in all examined OSCC specimens. *Ki-67*immunostaining as shown in figure (4) was observed as a nuclear stain restricted to the basal layer in healthy mucosae, whereas a broad positivity with variable distribution and intensity was found in the OSCC samples as shown in figures (5,6). Half of the cases (15 cases) were moderately proliferated score (++) and other (15 cases) were highly proliferated score (+++). Regarding correlation of two markers with the clinico- pathological findings of OSCC cases reveal that There was no significant correlation of these two markerswith the clinico- pathological findings(age, sex, tumor site, clinical presentation and histopathological grades).

Concerning Correlation between HOXA1 and Ki-67 expression score Table (3), result of present study revealed statistically significant positive correlation with P value = 0.027.

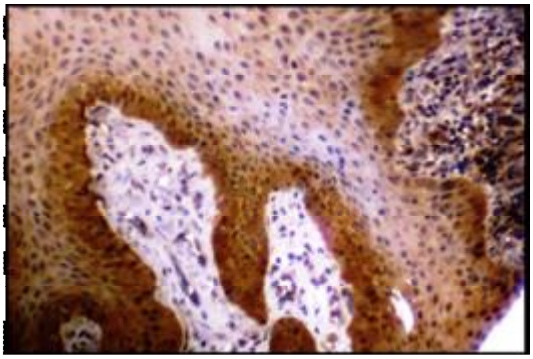

Figure 1: HOXA1 Expression in normal oral mucosa (10X).

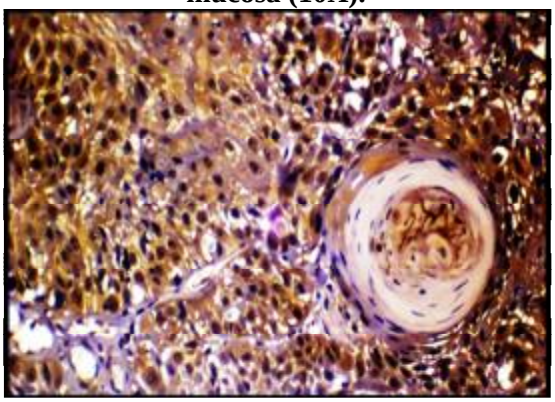

Figure 2: Positive expression of HOXA1 in well differentiated OSCC (10X).

Table 1: Clinico-pathological characteristics of 30 OSCC cases

	Frequency	Percent %
Age		
>50	21	70
24-50	9	30
Sex		
Male	21	70
Female	9	30
Tumor site		
Tongue	11	36.7
Maxilla	7	23.2
Mandibul	6	20
floor of mouth	2	6.7
buccal mucosa	2	6.7
Lip	2	6.7
Histological Grading		
Well	12	40
Moderate	13	43.3
Poor	5	16.7
Clinical Presentation		
Mass	22	73.3
Ulcer	6	20
White lesion	2	6.7

Table 2: HOXA1 expression in 30 cases of OSCC

HOXA1 score	Frequency	Percent
Score 1+	8	26.6%
Score 2+	11	36.7%
Score 3+	11	36.7%
Total	30	100%

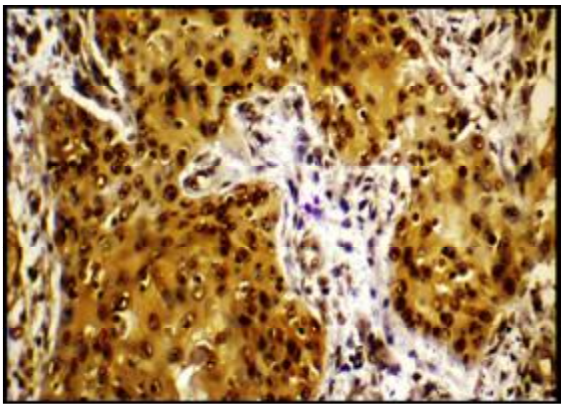

Figure 3: Positive expression of HOXA1 in moderate differentiated OSCC (10X).

Table 3: Correlation between HOXA1and Ki-67expression in OSCC.

HOXA1 score		Ki-67 score		Total		X^2	Sig.
		++	+++	N	%		
	1+	5	3	8	26.6	7.227	0.027 (S)
	2+	8	3	11	36.7		
	3+	2	9	11	36.7		
Total		15	15	30	100		

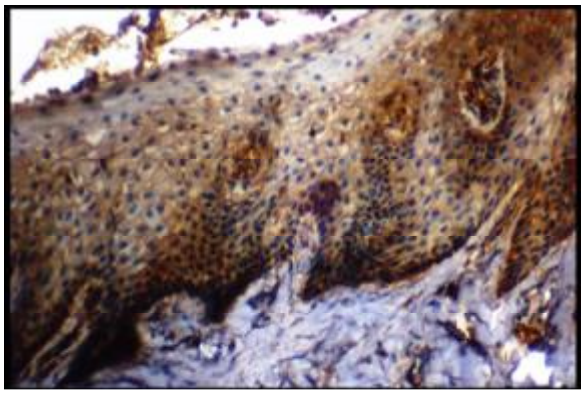

Figure 4: Ki-67 Expression in normal oral mucosa (10X).

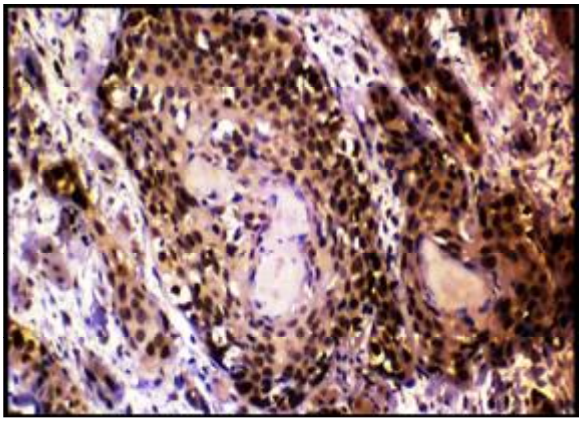

Figure 5: Positive expression of Ki-67 in well differentiated OSCC (10X).

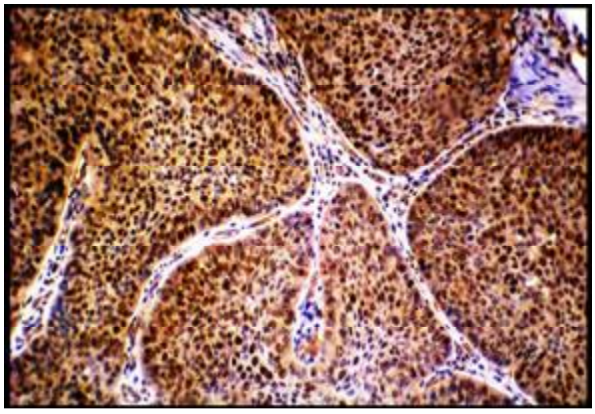

Figure 6: Positive expression of Ki-67 in moderately differentiated OSCC (10X).

DISCUSSION

Concerning the epidemiological parameters, including age, sex, site, clinical presentation, studies showed variable results; these inconsistent findings among different studies could be credit with the fact that the current study and some of the others are not an epidemiological type of studies, therefore the limited number and the random selection of the cases according to what is available preclude for definitive clinical findings.

Assessment of HOXA1 Immunohistochemistry

Immunoreactivity for *HOXA1*was observed as nuclear stain. Positive *HOXA1* expression was observed in all the studied cases of OSCC this finding was agreed with previous study [8] which was similar to this study.cytoplasmic staining was also observed in some cases with nuclear stain, this explain by interaction between *HOXA1* with numerous protein and transcription factors which was present primarily in the cytoplasm and involved in critical developmental process and then upon activation translocate to the nucleus to perform their function this interaction improved by study [9]. In normal oral mucosa, immuno staining was restricted to the basal and suprabasal layers only due to the fact that squamous epithelium keeps a continuous physiological regeneration in normal conditions, while broad positivity with variable distribution in OSCC sample, the intensity of *HOXA1expression* was found beyond basal localization suggests that a correlation between *HOXA1* expression and tumor progression may exist. Few studies[8]concerned *HOXA*1expression in OSCC which may be due to the fact that recently more attention has been paid to study this genes andTo our knowledgethis study is the first study in Iraq which demonstrates the *HOXA1* expression in OSCC particularly or other cancer.

However, many other studies [9-12] show expression of various members of *HOX* gene family in OSCC. Furthermore, aberrant expression of numerous *HOX* genes has been reported in various malignancies such as hematological malignancies [10] and variety of other solid tumors [13-15]

Regarding Correlation of HOXA1 expression with clinic-pathological parameters; this study revealed that *HOXA1* expression was not correlated with age, sex, clinical presentation and location of tumor. This finding was in agreement with previous study concerning OSCC [8], a non-significant correlation also was found concerning *HOXA1* expression and different tumor grades, opposite results were found by previous study [8]

Assessment of Ki-67 immunohistochemistry:

Cell proliferation is a biological process of vital importance and this control is lost in cancer [16]. Therefore, the knowledge of cellular proteins that control cell proliferation is essential for understanding tumor biology [17,18]. *Ki-67* antigen is a specific marker of proliferating cells [19].The IHC reactivity for NF Kb p65 was evaluated on the basis of presence or absence of brown nuclear and cytoplasmic staining (20) This study showed positive nuclear staining of *Ki-67* antigen in all OSCC cases and in all layers, whereas in normal oral mucosa positive *Ki-67* immunoreactivity was seen in the basal cell layer only (5). In addition, half of the positive cases showed high expression score and other half showed moderate expression score. Regarding the correlation of Ki-67 positive expression with age the results of the present study showed statistically non-significant correlation in *Ki-67* expression between the two age groups. This finding agreed with previous study [21] and disagreed with other [22]. Regarding the sex and sit of tumor there was statistically non-significant correlation. This is in accordance with [20-23], Also non-significant correlation was found in Ki-67 expression with different histopathological grades this finding was agree with some studies [1,24,25] and disagree with others [16,22,26,27] .

Correlations between HOXA1 and Ki-67 expression in OSCC

Uncontrolled cell proliferation plays a critical role in the development of a wide variety of carcinomas. Also it includes a very important cellular event in oral carcinogenesis that can be evaluated by immunohistochemical (IHC) study of abnormalities in cell cycle- regulatory proteins expression [28-30]. Regarding the correlation between both markers, the results revealed a significant correlation between them, this finding came in accordance with the previous study [8] and therefore suggesting their important role in oral tumorigenesis through ability of *HOXA1* to stimulate cell cycle progression and hence tumor development and progression.

REFERENCES

1. Bettendorf O, Piffko J, Bankfalvi A. Prognostic and predictive factors in oral squamous cell cancer: important tools for planning individual therapy? Oral Oncol 2004; 40: 110-9.
2. Massano J, Regateiro FS, Janurio G, Ferreira A. Oral squamous cell carcinoma: review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102:67-76.
3. Abate-Shen C. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer 2002; 2: 777-85.
4. Nunes FD, Almeida FC, Tucci R, Sousa SC. Homeobox genes: a molecular link between development and cancer. Braz Oral Res 2003; 17:94-8.
5. Humayun S, Ram Prasad VR. Expression of p53 protein and ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. Natl J MaxillofacSurg [serial online] 2011
6. Liu SC, Klein-Szanto AJ. Markers of proliferation in normal and leukoplakic oral epithelia. Oral Oncol 2000; 36:145-51.
7. Meer S, Galpin JS, Altini M, Coleman H, Ali H. Proliferating cell nuclear antigen and Ki67

immunoreactivity in ameloblastomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 213-21.
8. Bitu CC, Maria Destro MFDS, Carrera M, et al. HOXA1 is overexpressed in oral squamous cell carcinomas and its expression is correlated with poor prognosis. BMC Cancer 2012; 12:146 /1471-2407.
9. Vandeputte LJ, Remacle S, Bergiers I, et al. Protein interactions of the transcription factor Hoxa1. BMC Developmental Biology 2012; 12:29
10. Hassan NM, Hamada J, Murai T, Seino A, Takahas'hi Y, Tada M, Zhang X, Kashiwazaki H, Yamazaki Y, Inoue N, Moriuchi T. Aberrant expression of HOX genes in oral dysplasia and squamous cell carcinoma tissues. Oncol Res 2006; 16(5): 217-24.
11. Rodini CO, Xavier FCA, Batista K. Homeobox gene expression profile indicates HOXA5 as a candidate prognostic marke in oral squamous cell carcinoma. Int J Oncol 2012; 40 (4): 1180-8.
12. Yamatoji M, Kasamatsu A, Yamano Y, Sakuma K, Ogoshi K, Iyoda M, Shinozuka K, Ogawara K, Takiguchi Y, Shiiba M, Tanzawa H, Uzawa K. State of homeobox A10 expression as a putative prognostic marker for oral squamous cell carcinoma. Oncol Rep 2010; 23(1): 61-7.
13. Furuta J, Nobeyama Y, Umebayashi, et al. Silencing of Peroxiredoxin 2 and aberrant methylation of 33 Cp Gisland putative promoter regions in human malignant melanomas. Cancer Res 2006; 66: 6080-6.
14. Lewis MT. Homeobox genes in mammary gland development and neoplasia. Breast Cancer Res 2000; 2:158-69.
15. Carrio M, Arderiu G, Myers C, et al. Homeobox D10 indduces phenotypic reversion of breast tumor cells in a three-dimensional culture model. Cancer Res 2005; 65: 7177-85.
16. Tumuluri V, Thomas GA, Fraser IS. Analysis of the Ki-67 antigen at the invasive tumor front of human oral squamous cell carcinoma. J Oral Pathol Med 2002; 31: 598-604.
17. van Dierendonck JH, Wijsman JH, Keijzer R, van de Velde CJH, Cornelisse CJ. Cell-cycle-related staining patterns of antiproliferating cell nuclear antigen monoclonal antibodies comparison with BrdUrd labeling and Ki-67 staining. American Journal of Pathology 1991; 138 (5):1165-72
18. Tsuji T, Sasaki K, Kimura Y, Yamada K, Mori M, Shinozaki F. Measurement of proliferating cell nuclear antigen (PCNA) and its clinical application in oral cancers. Int J Oral MaxillofacSurg 1992; 21: 369-72.
19. Li TJ, Browne RM, Matthews JB. Expression of proliferating cell nuclear antigen (PCNA) and Ki-67 in unicystic ameloblastoma. Histopathology 1994; 26: 219-28.
20. Premalatha BR, Uma. Analysis of KI-67 antigen in human oral squamous cell carcinoma– An immunohistochemical study. J Int Oral Health 2010; 976 – 1799.
21. Issa HI. Immunoexpression of Epidermal Growth Factor Receptor, Ki-67 and P53 Protein in Squamous Cell Carcinoma of the Head and Neck. Pathology Department, Research Institute of Ophthalmology, Giza, Egypt, Consultant histopathology, King Khaled Civilian Hospital, Tabuk, KSA Research Journal of Medicine and Medical Sciences 2013; 8(1): 9-15.
22. Jassim ZM. Immune expression of cell cycle regulatory protein Ki-67, tumor suppressor gene P53 and Epstein barr virus proteins in oral squamous cell carcinoma. A Ph.D. thesis, Department of Oral Diagnosis, College of Dentistry, University of Baghdad, 2007.
23. Kuratomi K, Yano H, Tsuneoka M, Sakamoto K, Kusukawa J, Kojiro M**.** Immunohistochemical expression of Mina53 and Ki67 proteins in human primary gingival squamous cell carcinoma. Kurume Med J 2006; 53:71-8.
24. Roland NJ, Caslin AW, Bowie GL, Jones AS. Has the cellular proliferation marker Ki-67 any clinical relevance in squamous cell carcinoma of the head and neck. Clin Otolaryngol Allied Sci 1994; 19: 13-8..
25. Rolan P. The contribution of clinical pharmacology surrogates and models to drug development: a critical appraisal. Br J Clin Pharmacol 1997; 44: 219-25.
26. Tumuluri V, Thomas GA, Fraser IS. The relationship of proliferating cell density at the invasive tumor front with prognostic and risk factors in human oral squamous cell carcinoma. J Oral Pathol Med 2004; 33: 204- 8
27. Kurokawa H, Zhang M, Matsumoto S, Yamashita Y, Tanaka T , Tomoyose T, et al. The relationship of the histologic grade at the deep invasive front and the expression of Ki-67 antigen and p53 protein in oral squamous cell carcinoma. J Oral Pathol 2005; 34: 602-7.
28. Saito T, Nakajima T, Mogi K Immunohistochemical analysis of cell cycle-associated proteins p16, pRb, p53, p27 and Ki-67 in oral cancer and precancer with special reference to verrucous carcinomas. J Oral Pathol Med1999; 28: 226-32.
29. Adegboyega PA, Boromound N, Freeman DH. Diagnostic utility of cell cycle and apoptosis regulatory proteins in verrucous squamous carcinoma. Appl Immunohistochem. MolMorphol 2005; 13: 171-7.
30. Angadi PV, Krishnapillai R. Cyclin D1 expression in oral squamous cell carcinoma and verrucous carcinoma: correlation with histological differentiation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103, 30-5.