Immunohistochemical expression of Cyclin D1 and NF-KB p65 in oral lichen planus and oral squamous cell carcinoma (Comparative study)

Amer Mousa Abid, B.D.S. (1)
Muna S. Merza, B.D.S., M.Sc., Ph.D. (2)

ABSTRACT

Background: Oral Lichen Planus (OLP) is a chronic inflammatory mucosal disease, presenting in various clinical forms WHO had regarded OLP as a precancerous conditions in 1978 because of its potential with cancer. Both antigen-specific and nonspecific mechanisms involved in the pathogenesis of OLP. Oral Squamous Cell Carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity representing more than 94% of oral cancer. It occurs in different sites and has many etiological factors. Cyclin DI is a proto-oncogene which consider as the key protein in the regulation of cell proliferation and its overexpression led to the occurrence and progression of malignant tumors.NF-KB p65 is a member ofNF-kB family of transcription factors that widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival, also plays a major role in inflammation. The aims of this study were to evaluate the immunohistochemical expression of Cyclin D1 & NF-KB p65 in OLP & OSCC & to correlate the expression of the studied markers with the clinicopathological findings and with each other.

Materials and Methods: Fifty (50) formalin – fixed, paraffin – embedded blocks of both Oral Lichen Planus (25 cases) & Oral Squamous Cell Carcinoma (25 cases) were collected pro- and retrospectively were included in this study. Hematoxylin & Eosin stain was performed for each block for reassessment of histopathological examination. An immunohistochemical staining was performed using anti Cyclin D1 and anti NF-KB p65 monoclonal antibodies.

Results: Of twenty five OLPstudied cases , positive Cyclin D1 & NF-KB p65 expression was found in (84%) and (88%)of the cases respectively. For OSCC ,out of 25 studied cases ,positive Cyclin D1 & NF-KB p65 expression was observe in (88%) and (96%) of cases respectively. Statistically significant correlation between Cyclin D1immuno- reactivity and clinical presentation of OLP was found. Statistically significant correlation of Cyclin D1 immunoreactivity with tumor grade andNF-kBp65 immunoreactivity with tumor stage in OSCC cases was found. Statistically a highly significant correlation between the expression of two studied markers in OLP and OSCC was found.

Conclusion: A highly significant correlation was seen regarding the expression of both markers with each other, suggesting their cooperative role in the pathogenesis of OLP and OSCC.

Keywords: OLP, OSCC, Cyclin D1, NF-KB p65, Immunohistochemistry. **(J Bagh Coll Dentistry 2014; 26(1):80-87).**

INTRODUCTION

Oral Lichen Planus is a relatively common chronic inflammatory disease of oral mucosa with a prevalence rate of 0.5% - 2.2% of the population. Clinically, OLP may assume a variety of morphological changes (1). The premalignant potential of OLP is still debatable. Malignant transformation has been estimated to occur in 0.5 – 2.9% of the OLP patients. Currently, there are no prognostic markers to identify which chronic OLP lesions are at a higher risk for progression. Thus, every OLP patient should be monitored carefully to detect early cancer development (2).

Oral Squamous Cell Carcinoma is the most commonly diagnosed oral cancer(3).It is the malignancy of stratified squamous epithelium. It remains a lethal disease in over 50% of the cases diagnosed annually, due mostly to late detection of advanced cancer (4). Studying its molecular pathway may help in searching for molecular markers that might predict the clinical behavior of the tumor, which may not strictly related to TNM staging or histological grade (5).

(1) M.Sc. Student, Department of Oral and maxillofacial pathology, College of Dentistry, University of Baghdad
(2) Assistant Professor, Department of Oral and maxillofacial pathology, College of Dentistry, University of Baghdad

Cyclin D1, a 45 kD (kilo Dalton) protein encoded by Cyclin D1 gene (CCND1) located on chromosome 11q13, is a part of the molecular system that regulates the cell cycle G1 to S transition (6). Overexpression of Cyclin D1 leads to shortening of G1 phase and less dependency on growth factors resulting in abnormal cell proliferation which in turn might favour the occurrence of additional genetic lesions (7). Cyclin D1 expression has been studied in various carcinomas including oral squamous cell carcinomas. Some studies have been carried out to correlate the expression of Cyclin D1 with histological grading of this neoplasm (8).Nuclear Factor-kappa B is a transcription factor that induces the expression of various genes, leading to inflammatory reactions, embryonic morphogenesis, and anti apoptosis. In recent years studies suggested that NF-κB has been implicated in the regulation of cell proliferation, transformation, and tumor development. NF-κB was found to stimulate transcription of cyclin D1, a key regulator of G1 checkpoint control. NF-κB binding site in the human Cyclin D1 promoter conferred activation by NF-κB as well as by growth factors. Both levels and kinetics of CyclinD1 expression during G1 phase were

controlled by NF-κB. Recent advances in biological research have revealed that NF-κB can stimulate neovascularization[9]. More ever continuous NF-kB activity protects cancer cells from apoptosis and in some cases stimulates their growth. Therefore, many current anti tumor therapies seek to block NF-kB activity as a means to inhibit tumor growth or to sensitize the tumor cells to more conventional therapies, such as chemotherapy[10].

This study aimed to:-

- Evaluate immunohistochemical expression of (Cyclin D1) and (NFkBp65) monoclonal antibodies in oral lichen planus and oral squamous cell carcinoma.
- Correlate the expression of the studied markers with the clinicopathological findings of OLP and OSCC.
- Correlate the expression of the studied markers with each other.

MATERIALS AND METHODS

Twenty-five cases , histologically diagnosed as oral lichen planus and other twenty –five cases, histologically diagnosed as oral squamous cell carcinoma were collected pro- and retrospectively from the archives of Oral Pathology Department , College of Dentistry, Baghdad University, Al-shaheed Ghazi hospital and Teaching laboratories Department , Medical city/ Baghdad from (2004 – 2011). The clinicopathological information regarding age, sex, tumor site, clinical presentation, tumor grading and staging was obtained from the case sheets presented with the specimens. The clinico-pathological characteristics of OLP and OSCC patients from which the specimens were taken are illustrated in table 1 and 2 respectively.

Four µm thick sections were cut and hematoxylin and eosin slides were prepared for histopathological reassessment. Another 4 µm thick sections were cut for immunoshitochemcial staining with anti Cyclin D1 and anti NFkBp65 monoclonal (Abcam UK)). Negative and positive controls were included in each IHC run.Tissue blocks of small intestine were used for Cyclin D1 and breast adenocarcinoma blocks were used for NFkBp65 (according to antibodies manufacturer). For immunohistochemistry, the sections were mounted on positively charged slides. Slides were baked in hot air oven at 65°C overnight.

Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps.

Table 1: Clinicopathological findings of OLP cases

Age	**Frequency**	**%**
20-40	9	36
41-60	14	56
age>60	2	8
Total	25	100
Sex	**Frequency**	**%**
Male	9	36
Female	16	64
Total	25	100
Site	**Frequency**	**%**
buccal mucosa	23	92
lower lip	2	8
Total	25	100
Presentation	**Frequency**	**%**
White lesion	18	72
Red lesion	4	16
White &red lesion	3	12
Total	25	100

Table 2:Clinicopathological findings of OSCC cases

Age	**Frequency**	**%**
20-40	3	12
41-60	12	48
age>60	10	40
Total	25	100
Sex	**Frequency**	**%**
Male	15	60
Female	10	40
Total	25	100
Site	**Frequency**	**%**
tongue	11	44
buccal mucosa	7	28
lower lip	2	8
alveolar ridge	5	20
Total	25	100
Presentation	**Frequency**	**%**
White lesion	2	8
Mass	9	36
Ulcer	14	56
Total	25	100
Grade	**Frequency**	**%**
Well	8	32
Mod	14	56
Poor	3	12
Total	25	100
Stage	**Frequency**	**%**
I	6	24
II	3	12
III	7	28
IV	9	36
Total	25	100

Antigen (Ag) retrieving was done for both markers as recommended by the manufacturer. Then endogenous peroxidase activity was blocked followed by blocking the non- specific staining. Primary Abs (100 ml) were applied for each section. Cyclin D1 and NF kappa B p65 were diluted into 1/1000 & 1/250 respectively.

After an overnight incubation and washing with phosphate buffered solution (PBS), secondary Abs were applied, incubated and rinsed with a stream of PBS. Primary Abs were visualized with 3,3-diaminobenzidine (DAB) chromogen, then counterstained with Mayer's hematoxyline, dehydrated and mounted.

Evaluation of Immunohistochemistry Results:

In both OLP and OSCC, the cells with clear brown **nuclear** staining were considered positive for Cyclin D1 immunostaining whereas cells with clear brown **cytoplasmic** staining were considered positive for NF kappa Bp65 immunostaining within a violet-blue tissue section background of Hematoxyline staining. The immunoreactivity in tumor cells was classified and scored as follows: - CyclinD1, Negative (-),(1+) < 1% , (2+) 1-25% , 26-50%, (3+) 50-75% and (3+) >75% (11). For NF kappa Bp65, negative (-) no detectable immunostaining or basal immunostaining in<10%, (1+) mild immunostaining 10-30%.(2+) moderate immunostaining 30-50%. (3+) strong immunostaining >50%.(12).

Statistical Analysis

Numerical values were used in this study for describing the variables which includes: No. mean, SD for age, cyclin D1 and NF kappa B p65. Categorical variable which includes: sites, grade, gender and clinical presentation were described using no. and percentage.

Pearson correlation coefficient of correlation (r) was used to find the relation between two markers. ANOVA test (analysis of variance) was used to detect differences for age and two markers .Chi-square test the relationship between categorical variables. Statistical analysis was done using SPSS (statistical package for social sciences) V17 (2008).

RESULTS

Immunostaining of Cyclin D1 was detected as a brown staining in the nucleus of target antigen cells, in OLP cases positive IHC Cyclin D1expression was found in 21 cases (84%),of which, 9cases (36%) showed score 2 immunostaining, 6 cases (24%) with score 3 , 4 cases (16%) score1 and 2cases (8%) score 4. Figures (1,2). In OSCC positive Cyclin D1 IHC expression was found in 22 cases (88%), of which, 7cases (28%) with score 2 immunostaining, followed by 6 cases (24%) score 3, 5 cases (20%) score 4and 4cases (16%) with score1 .Figures (3, 4, 5)

Correlating the positive expression of Cyclin D1 with the clinico- pathological findings of OLP revealed statistically significant correlation with clinical presentation (p value= 0.025) , whereas non significant correlation was observed with other clinico- pathological parameters. Table(3). In OSCC, results revealed statistically significant correlation regarding Cyclin D1 expression in relation to tumor grade (p value =0.021),Table(4).While, nonsignificant correlation was observed with other clinico-pathological parameters.

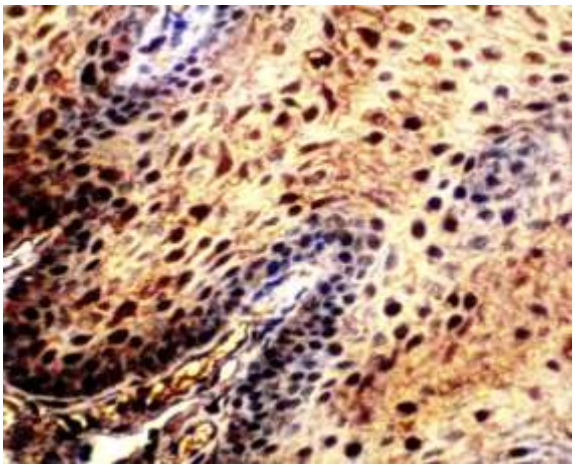

Figure 1: Positive brown nuclear immunostaining of Cyclin D1 in OLP (400X)

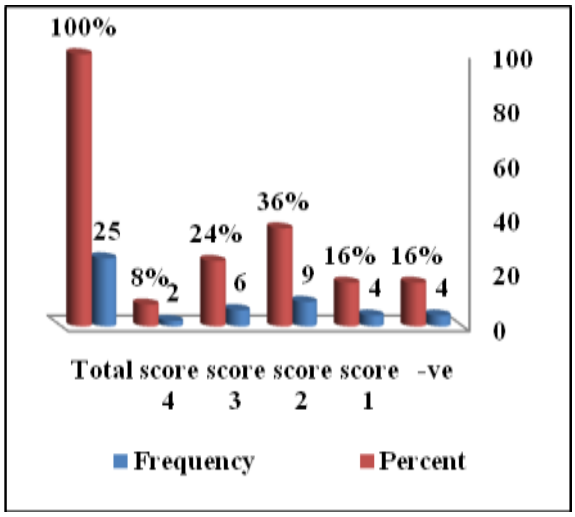

Figure 2: Percentage frequency distribution of Cyclin D1 expression in OLP cases

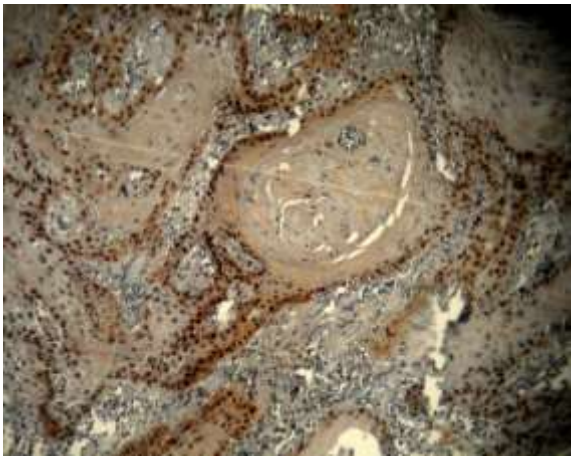

Figure 3: Positive brown nuclear immunostaining of Cyclin D1 in well differentiated OSCC (100X)

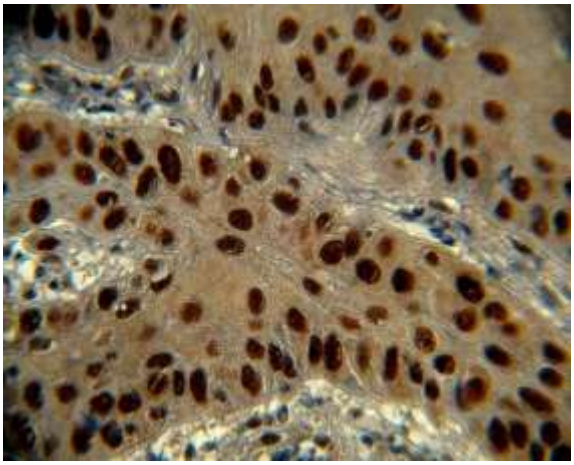

Figure 4: Positive brown nuclear immunostaining of Cyclin D1 in poorly differentiated OSCC (400X)

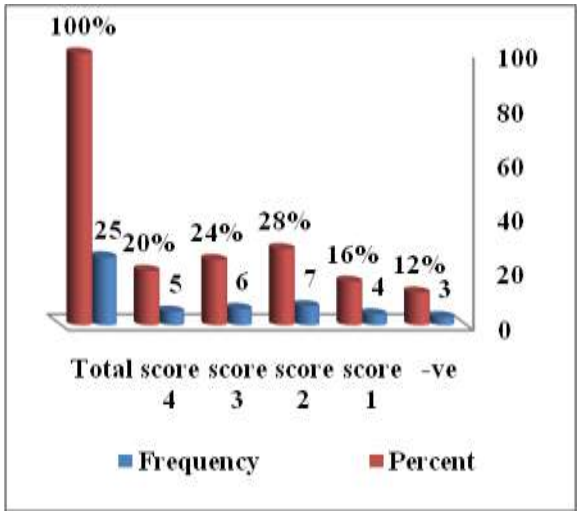

Figure 5: Percentage frequency distribution of Cyclin D1 expression in OSCC cases

Immunostaining of NF-kBp65 was detected as a brown staining in the cytoplasm of target antigen cells. In OLP cases, positive IHC expression was found in 22 cases (88%), of which, 6 cases (24%) showed strong immunostaining followed by 9 cases (36%) showed moderate immunostaining and 7cases (28%) with mild immunostaining Figures(6 ,7). In OSCC, Positive NF-kBp65 IHC expression was found in 24 cases (96%), of which, 6 cases (24%) showed strong immunostaining followed by10cases (40%) with mild immunostaining and 8 cases (32%) showed moderate immunostaining . Figures (8, 9, 10)

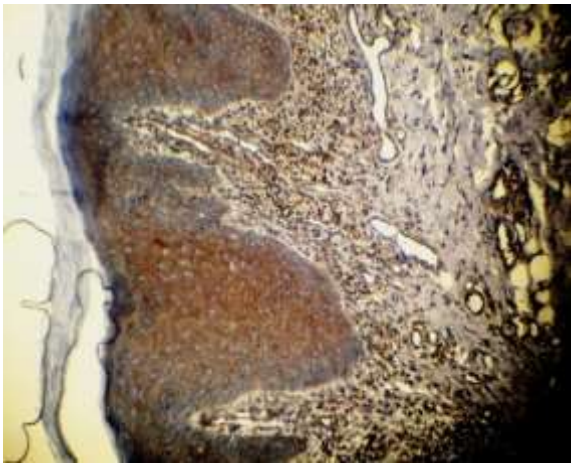

Figure 6: Positive brown cytoplasmic immunostaining of NF-kBp65 in OLP (100X)

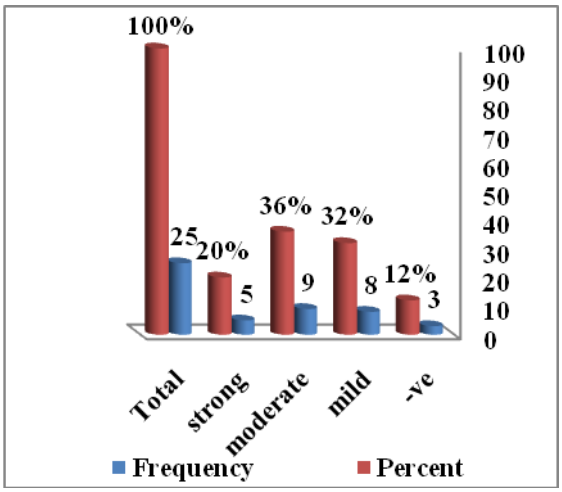

Figure 7: Percentage frequency distribution of NF-kBp65 expression in OLP cases

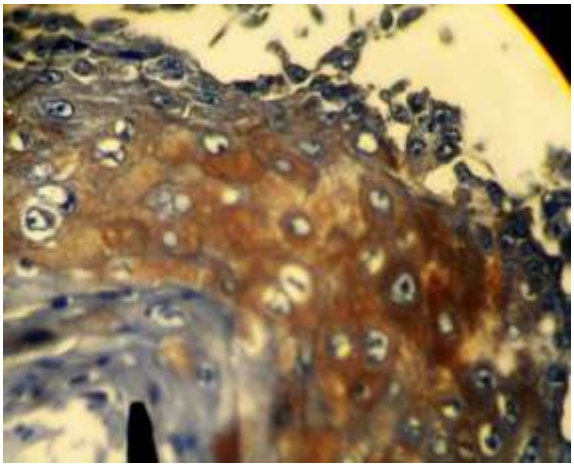

Figure 8: Positive brown cytoplasmic immunostaining of NF-kBp65 in well differentiated OSCC (400X)

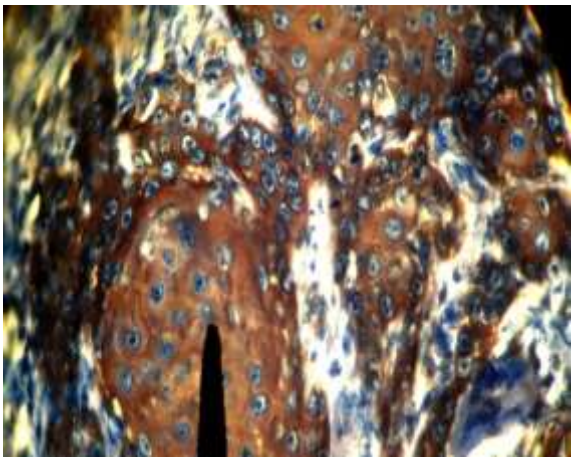

Figure 9: Positive brown cytoplasmic immunostaining of NF-kBp65 in moderately differentiated OSCC (400X)

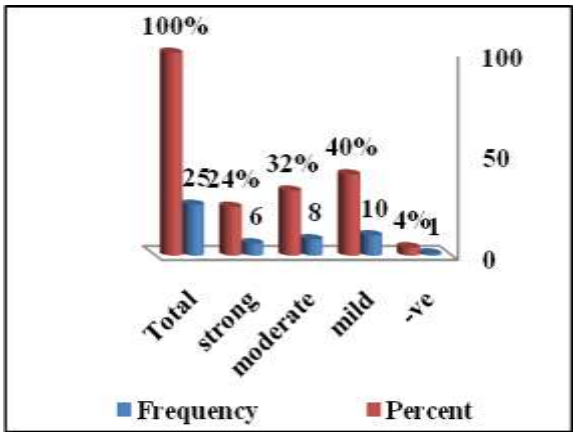

Figure 10: Percentage frequency distribution of NF-kBp65 expression in OSCC cases

Correlating NF-kBp65positive expression with the clinico- pathological findings of OLP cases revealed statistically non significant correlation. While in OSCC there was statistically significant correlation with tumor stage (p value =0.037). Table (5). On other hand, non significant correlation was observed with other clinico- pathological parameters.

Table 3: Correlation of Cyclin D1 with clinical of OSCC cases

Presentation		White lesion	Red lesion	White and red	Total
CD1 scoring	0	4	0	0	4
	1	4	0	0	4
	2	7	0	2	9
	3	3	2	1	6
	4	0	2	0	2
Total		18	4	3	25
Pearson Chi-Square			Value	df	Asymp. Sig. (2-sided)
			17.515	8	0.025

Table 4: Correlation of Cyclin D1 with tumor grade of OSCC cases

Grade		Well	Mod	Poor	Total
CD1 scoring	0	3	0	0	3
	1	3	1	0	4
	2	1	6	0	7
	3	1	4	1	6
	4	0	3	2	5
Total		8	14	3	25
Pearson Chi-Square			Value	df	Asymp. Sig. (2-sided)
			18.035	8	0.021

Table 5: Correlation of NF-kBp65 with tumor stage of OSCC cases

Stage		I	II	III	IV	total
NF scoring	0	1	0	0	0	1
	1+	5	1	2	2	10
	2+	0	2	4	2	8
	3+	0	0	1	5	6
Total		6	3	7	9	25
Pearson Chi-Square			Value	df	Asymp. Sig. (2-sided)	
			17.824	9	0.037	

Concerning the correlation of Cyclin D1 and NF-kBp65 with each other, results of present study observed statistically a highly significant correlation between the expression of two markers in both OLP (p value =0.007) and OSCC (p value =0.003).Tables (6 ,7).

Table 6: Correlation of Cyclin D1 withNF-kBp65 expressions in OLP

		NF-KB
CyclinD1	Pearson Correlation	.526**
	Sig. (2-tailed)	0.007
	N	25
**. Correlation is significant at the 0.01 level (2-tailed).		

Table 7: Correlation of Cyclin D1 withNF-kBp65 expressions in OSCC

		NF-KB
CyclinD1	Pearson Correlation	.563**
	Sig. (2-tailed)	0.003
	N	25
**. Correlation is significant at the 0.01 level (2-tailed).		

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 CyclinD1 Immunohistochemistry

Cyclin D1 is a positive regulator of cell cycle, and it exerts its effects on the Rb pathway, lead to release the E2F transcription factors thereby allowing the cells to enter the S phase. Overexpression of Cyclin D1 may lead to shortening of G1 phase, increased cell proliferation and reduced dependency on growth factors**.** This may contribute to disturbance in the normal cell cycle control and mitogenic signaling pathways enhancing the cell transformation and tumorogenecity. Thus, overexpression of Cyclin D1 is thought to provide the tumor cells with a selective growth advantage [13].

In Oral Lichen Planus ,results of present study showed positive Cyclin D1expression in 21 cases (84%) of OLP studied cases.This finding was agree with [13,14] who found Cyclin D1 positive expression in (71.67%) and (82%) respectively . This finding suggests that Cyclin D1 may play important role in the occurrence , progression and carcinogenic process of OLP [14].

In OSCC, 22 of 25 cases (88%) showed positive immunoreactivity to Cyclin D1. This finding was in concordance with previous studies [15,16] who reported positive expression of Cyclin D1 in (85.7%) and (95%) respectively. These studies support the role of Cyclin D1 as a potential marker of proliferation and oncogenesis.

Assessment ofNF-kBImmunohistochemistry

NF-kB is a signal transcription factor found to play an important role in aberrant gene expression and the malignant phenotype of SCC, other studies revealed that expression of a very large number of genes is stimulated by NF-kB. These genes encode members of various classes of proteins that are involved in the inflammatory response such as cytokines, HLA molecules, tumor necrosis factor, cellular adhesion molecules, acute phase proteins, and growth factors [17].

In Oral Lichen Planus, 22 (88%) of OLP studied cases showed positive immunoreactivity to NF-kBp65.This finding was agree with [13,18] who found NF-kBp65 positive expression in (85%) and (93%) of OLP cases respectively. Similarly, [19] found positive NF-kBp65-expression in (68%) of oral premalignant cases. In previous Iraqi study, positive NF-kappa Bp65 expression was found in 83.8% of the studied oral dysplastic lesions with low expression score observed in normal oral mucosa indicating the role of this factor in transition from normal oral mucosa to premalignant lesions mostly through prevention of apoptosis [20].

In Oral Squamous Cell Carcinoma, the results of present study showed positive immunoreactivity to NF-kBp65in 24 of 25 cases (96%). This finding come in accordance with[20,21], who found that NF-kBp65was expressed in (100%) and (80%) of OSCC cases respectively. Moreover, [19,22] also found that NF kBp65was expressed (68%) & (78.38%) of OSCC cases respectively. Increased activation of NF-kB has been shown to occur in premalignant dysplastic lesions and approximately 85% of HNSCCs, indicating it is an early event in carcinogenesis and the association of strong immunostaining with increased rate of malignant progression of dysplasia might shed light on molecular basis of gene therapy of oral cancer [23,24].The variations observed in the immmunopositivity rate among different studies could be due to the antibodies used and the processing techniques performed, as well as the heterogeneity of OLP&OSCC which could be attributed to the epidemiological or biological differences between countries and population.

Assessment of the correlation between Cyclin D1and NF-kBp65 IHC expression in OLP and OSCC

NF-κBp65 was found to stimulate transcription of cyclin D1, a key regulator of G checkpoint control. Two NF-κB binding sites in the human Cyclin D1 promoter conferred activation by NF-

κB as well as by growth factors. Both levels and kinetics of Cyclin D1 expression during G phase were controlled by NF-κB. Moreover, inhibition of NF-κB caused a pronounced reduction of serum-induced cyclin D1-associated kinase activity and resulted in delayed phosphorylation of the retinoblastoma protein [25].

It has been reported that on one hand Cyclin D1can inhibits NF-κB transcriptional activity through a co repressor function [26],and on the other hand , NF-κB is activate the transcription of cyclin D1gene,which then increase the expression of Cyclin D1 protein [27]. Furthermore, [28] found that the regulation of Cyclin D1 transcription by Ral GTPases is dependent on NF-κB activation and is mediated through an NF-κB binding site in the Cyclin D1 promoter.Despite variances in mechanism, it is well established that NF-κB are strong inducers of Cyclin D1 gene expression [29-31]. The present study showed statistically significant correlation between Cyclin D1 and NF-κB in OLP & OSCC cases. Similarly, [22] also found a correlation between Cyclin D1 and NF-κB in oral leukoplakia and oral squamous cell carcinoma, Moreover he reported that there was a significant correlation between the expression of NF-κB/p65 and Cyclin D1 in the cancerization of oral leukoplakia, which played a cooperative role in cell proliferation and took part in the carcinogenesis of OSCC.

Finally, the statistically significant correlation between Cyclin D1 and NF-κB in OLP & OSCC cases observed in this study suggests their close and synergistic, cooperation and coactivation in premalignant & malignant lesions, Therefore, they could be considered important biomarkers acting together for evaluating the malignant potential of OLP.

REFERENCES

1. Neville Brad W, Douglas D, Carl M, Jeery E. Oral Maxillofacial Pathology. 3rd ed. Saunders; 2009.
2. Sugerman P, Savage N, Walsh L, Zhao Z, Zhou X, Khan A, Seymour GJ, Bigby M. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 2002; 13(4): 350-65.
3. Vermorken JB, Remenar E, vanHerpen C, Gorlia T, Mesia R, Degardin M. Cisplatin fluorouracil and docetaxel in unresectable head and neck cancer. N Engl J Med 2007; 357:1695-1704.
4. Warnakulasuriya. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45:309–16. (IVSL)
5. Abbas NF, EL- Sharkawy SL, Abbas EA, EL- Shaer M. Immunohisto-chemical study of P53 and angiogenesis in benign and preneoplastic oral lesions and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103(3): 385-90.
6. Punnya V, Rekha K. Cyclin D1 expression in oral squamous cell carcinoma and verrucous carcinoma: correlation with histological differentiation. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 2007; 103(3): e30-e35.
7. Todd R, Hinds PW, Munger K,Rustgi AK, Opitz OG, Seliman Y. Cell cycle disregulation in oral cancer. Crit Rev Oral Biol Med 2002; 13(1): 51-61.
8. Mishra R, Das B. CyclinD1 expression and its possible regulation in chewing tobacco mediated oral squamous cell carcinoma progress- ion. Archives Oral Biol 2009; 54(10): 917–23.(IVSL)
9. Campbell K, Perkins N. Regulation of NF-kappaB function. BiochemSocSymp 2006; 73:165^80.
10. Beyaert R. Nuclear Factor-kappa B: Regulation and Role in Disease. Kluwer Academic Publishes, Dordrecht, The Netherlands 2004; 426.
11. Gu Y, Zhang S, Wu Q, et al. Differential expression of decorin, EGFR and cyclin D1 during mammary gland carcinogenesis in TA2 mice with spontaneous breast cancer. Journal of Experimental and Clinical Cancer Res 2010; 22: 29– 36.
12. Sawhney M, Rohatgi N, Kaur J, Shishodia S, Sethi G, Gupta SD, Deo SVS, Shukla NK, Aggarwal BB, Ralhan R. Expression of NF-jB parallels COX-2 expression in oral precancer and cancer: Association with smokeless tobacco. Int J Cancer 2007; 120: 2545–56.
13. Yao X, Yin C, Shen L, Xie S. Expressions of NF--kappaBp65, TRAF2, cyclinD1 and their association with cell apoptosis in oral lichen planus. J Southern Medical University 2007; (11): 1657-60
14. Zhang Z, Chen L, Hong Q, Sun S. Expression of TGF-β1,Smad 4 and Cyclin D1 in oral lichen planus. Chinese J Conser Dent 2010.
15. Adriana N, Ricardo M, Moacir N, Edna T, Suzana O, Machado S. Comparison between Immunohisto-chemical Expression of Cyclin D1 and p21 and Histological Malignancy Graduation of Oral Squamous Cell Carcinomas. Braz Dent J 2004; 15(2): 93-8. (IVSL)
16. Swaminathan U, Joshua E, Rao U, Ranganathan K. Expression of p53 and Cyclin D1 in oral squamous cell carcinoma and normal mucosa: An Immunohistochemical study. J Oral Maxillofac Pathol 2012; 16:172-7
17. Van Waes C .Nuclear factor-kappa B in development, prevention, and therapy of cancer. Clin Cancer Res 2007; 13(4):1076–82
18. Shi YJ, Shen LJ, Yin C.Expression of caspase-8, receptor interacting protein and nuclear factor-kappaBp65 in oral lichen planus. Chinese J Stomatol 2010; 45(1):11-5
19. Meenakshi S, Nidhi R, Jatinder K, Shishir S, Gautam S, Siddhartha D, Suryanaryana V, Nootan K, Bharat B ,Ranju R.Expression of NF-kB parallels COX-2 expression in oral precancer and cancer: Association with smokeless tobacco. Int J Cancer 2007; 120: 2545–2556.(IVSL)
20. Merza MS. Differential expression of intracellular apoptotic signaling molecules (Procaspase- 3, Fas, Fas ligand, Nuclear factor KB and C-myc) in dysplasia and squamous cell carcinoma of oral mucosa by in situ technique. A Ph.D. thesis in Oral Pathology, College of Dentistry, University of Baghdad, 2006.

21. Bindhu O, Ramadas K, Paul Sebastian, RadhakrishnaPillai M. High expression levels of nuclear factor kappa B and gelatinases in the tumorigenesis of oral squamous cell carcinoma. Head Neck 2006; 28(10): 916-25.
22. Jiang Q. Expression of NF-κB,Cyclin D1 and CD105 in oral leukoplakia and oral squamous cell carcinoma. Chinese paper 2007; (31)
23. Zhang PL, Pellitteri PK, Law A, Gilroy PA, Wood GC, Kennedy TL, Blasick TM, Lun M, Schuerch C III, Brown RE . Overexpression of phosphorylated nuclear factor-kappa B in tonsillar squamous cell carcinoma and high-grade dysplasia is associated with poor prognosis. Mod Pathol 2005; 18(7):924–932.
24. Allen C, Saigal K, Nottingham L, Chen Z, Van Waes C. Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative NF-kappaB subunits or other prosurvival signal pathways activated in head and neck cancer. Clin Cancer Res 2008; 14(13): 4175–85
25. Michael H, Daniel K, Alexandra E, Andreas H, Claus S , Michael S. NF-κB Function in Growth Control: Regulation of Cyclin D1 Expression and G /G -to-S-Phase Transition. Mol Cell Biol 1999; 19(4): 2690-8.
26. Maria F, Pablo N , Cecilia V, Panelo L, Marina R , Georgina P, Giselle A , Sabrina M. Cyclin D1 is a NF-κB corepressor. Biochimica et Biophysica Acta 2012; 1823: 1119–31
27. Chen F, Castranova V. Nuclear factor-kappaB, an unappreciated tumor suppressor. Cancer Res 2007; 67:11093-11098.
28. Dale O, Serge A, Kiran J, Maofu F, Richard G, Jacques H, Michael A.Ral GTPases Contribute to Regulation of Cyclin D1 through Activation of NF-κB. Mol Cell Biol 2000; 20: 8084-92
29. MatosP, JordanP. Expression of Rac1b stimulates NF-kappaB-mediated cell survival and G1/S progression. Exp. Cell Res 2005; 305: 292-9.
30. Klein EA, Yang C, Kazanietz MG,Assoian RK.NFkappB -independent signaling to the Cyclin D1 gene by Rac. Cell Cycle 2007; 6: 1115-1121.
31. Yang C, Klein EA, Assoian RK and Kazanietz MG.Heregulin beta1 promotes breast cancer cell proliferation through rac/erk-dependent induction of Cyclin D1 and p21 Cip1. Biochem J 2008;410:167-75