An Immunohistochemical Expressions of BAD, MDM2, and P21 in Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a common malignancy characterized by poor prognosis and low survival rate. The purpose of this study was to evaluate the Immunohistochemical expressions of BAD, MDM2, and P21as apoptotic markers in oral squamous cell carcinoma.

Materials and methods: This study was performed on forty formalin-fixed paraffin-embedded blocks which histopathologically diagnosed as Oral Squamous Cell Carcinoma. All cases were collected from the Histopathological Laboratory from patients treated surgically at Maxillofacial surgery Department at Ramadi Teaching Hospital, Iraq.

Results: The immunohistochemical staining of BAD showed positive expression in 39 (97.5%), MDM2 showed positive expression in 39(97.5%) and P21showed positive expression in 34(85%) of the collective cases.

Conclusion: A statistically significant correlation was found regarding MDM2 with the tumor site, P21 with tumor grade.

Keywords: Squamous cell carcinoma, Metastasis, Biomarkers, Carcinogenesis, Apoptosis. (J Bagh Coll Dentistry 2016; 28(2):34-39).

INTRODUCTION

Oral Squamous cell carcinoma (OSCC) is a malignant neoplasm of invasive stratified squamous epithelium with varying degrees of squamous differentiation [1]. It is capable of locally destructive growth, extensive lymph node invasive and distant metastasis. More than 90% of malignant neoplasms of the oral cavity and oropharynx are squamous cell carcinomas of the lining mucosae with relatively rare neoplasms arising in minor salivary glands and soft tissues[2].

The Bcl-2-associated death promoter (BAD) protein is a pro-apoptotic member of the Bcl-2 gene family which is involved in initiating apoptosis [3]. When BAD is phosphorylated by Akt/protein kinase B, it forms the BAD-protein homodimer. This leaves Bcl-2 free to inhibit Bax-triggered apoptosis [4].

MDM2 is a protein that normally inhibits the function of p53 by causing its degradation. If the DNA damage is repaired successfully, quite ingeniously, p53 activates MDM2, whose product binds to and degrades p53, thus relieving the cell-cycle block [5].

P21 is a potent cyclin-dependent kinase inhibitor (CKI). The p21 protein binds to and inhibits the activity of cyclin-CDK2, -CDK1, and -CDK4/6 complexes, and thus functions as a regulator of cell cycle progression at G1 and S phase. In addition to growth arrest, P21 can mediate cellular senescence [6] and it interacts with proliferating cell nuclear antigen (PCNA) and plays a regulatory role in S phase DNA replication and DNA damage repair, sometimes p21 is expressed without being induced by p53. This kind of induction plays a big role in p53 independent differentiation which is promoted by p21 [7].

MATERIALS AND METHODS

The sample of the present study was forty formalin-fixed paraffin-embedded blocks of OSCC cases. All were collected from the Histopathological Laboratory at Maxillofacial Surgery Department at Ramadi Teaching Hospital. Demographical and clinical data provided by surgeon were obtained from the case sheets presented with tumor specimens, including information concerning patient's name, age, gender, clinical presentation, site of tumor, lymph node involvement, distant metastasis (if present).

Each formalin- fixed paraffin-embedded specimen had serial sections prepared as follows: 5µm thickness sections were mounted on glass slides for routine Haematoxylin and Eosin staining (H&E), from each block of the studied sample and the control group for Histopathological evaluation.

Three sections of 5µm for positive and negative tissue and technical control were taken and mounted on positively charged microscopic slides (Biocare medical USA and Afco brand China) to obtain a greater tissue adherence. H & E staining was used for reassessment of histopathological examination of the collected samples and control group. For each specific antibody (BAD, MDM2, and P21, Abcam-USA)

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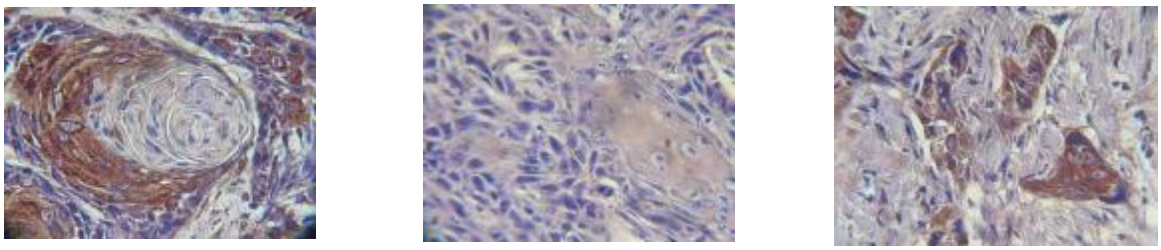
the recommended dilution was applied (1/80, 1/50, and 1/250 respectively).

Specific expression was demonstrated by the absence of immunostaining in the negative control slides and its presence in recommended positive controls. Any positivity in the examined slides for tumor cells the case consider positive, while if no positive expression where noted the case considered negative. The expression for all markers was evaluated semi-quantitatively. It was obtained by counting the number of tumor cells in 5 fields (using 40X objective in most represented areas of sections) and calculate the percentage of tumor cells that labeled as brown cytoplasmic and/or nuclear staining pattern (according to type of expression for each marker).

Labeling index for each field was calculated using the following equation: (number of positive cells/ number of total cells); the mean value of labeling indices for the five fields was considered to be the label index for the case. The scoring was done under light microscope and assigned to four categories:

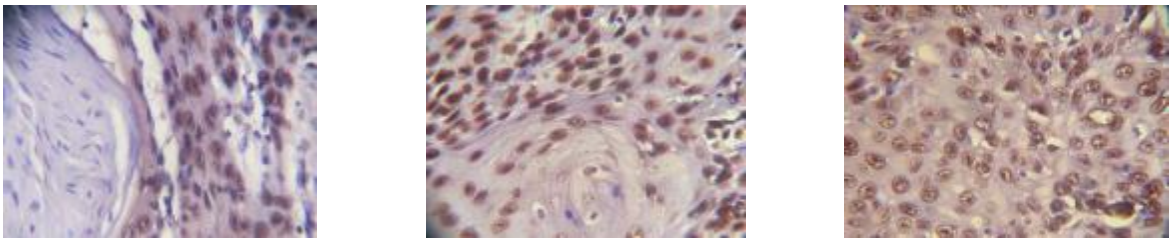
- **No Expression (NE)** = 0 expression,
- **Mild (MI)** = 1-20 expression,
- **Moderate (MO)** = 20-50 expression,
- **Strong (ST)** = 50-100 expression (Figures 1, 2 and 3).

Chi-square test was applied for statistical assessment of clinicopathological and immunohistochemical findings to identify the significant or non-significant correlation between them at 95% confidence interval.



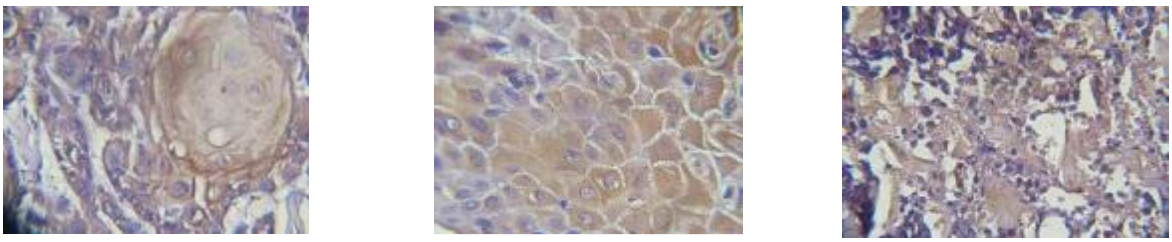
A. Positive cytoplasmic expression in WD (40X) **B. Positive cytoplasmic expression in MD (40X)** **C. Positive cytoplasmic expression in PD (40X)**

Figure 1: A, B and C Immunohistochemical pattern of expression of BAD



A. Positive nuclear expression in WD (40X) **B. Positive nuclear expression in MD (40X)** **C. Positive nuclear expression in PD (40X)**

Figure 2: A, B and C Immunohistochemical pattern of expression of MDM2



A. Positive cytoplasmic expression in WD (40X) **B. Positive cytoplasmic expression in MD (40X)** **C. Positive cytoplasmic expression in PD (40X)**

Figure 3: A, B and C Immunohistochemical pattern of expression of P21

RESULTS

Forty cases of OSCC were included in the present study with age range between 20-85 years old and mean age 52.4 years old, including 26(65%) males and 14(35%) females. The total positive immunohistochemical expression of BAD was found in 39 (97.5%) of the cases and as follows; Strong in 13(32.5%) cases, moderate expression in 20(50%) cases and mild expression in 6(15%) cases; while negative expression was found in 1(2.5%) case. Concerning the anatomical site and according to the number of the cases included in the present study, the recorded percentage of immunohistochemical expression of BAD in the lower lip was positive moderate expression in 24(60%) cases.

The highest percentage of positive BAD immunohistochemical expression was in well differentiated SCC as seen in 17(42.5%) cases, followed by moderately differentiated SCC as seen in 16(40%) case. An equal positive BAD immunohistochemical expression percentage was recorded in stage I & II 14(35%) cases for each and 8(20%) cases in stage III (Table 1).

The total MDM2 positive immunohistochemical expression was found in 39(97.5%) of the cases; including strong expression in 17(42.5%) cases, moderate expression in 17(42.5%) cases and mild expression in 5(12.5%) cases and negative expression was found only in 1(2.5%) case.

Most of the positive cases were located in lower lip in 24(60%) cases followed by the alveolus in 7(17.5%) of the cases. The highest percentage of positive MDM2 immunohistochemical expression in well differentiated SCC in 18(45%) cases, followed by moderately differentiated SCC in 16(40%) case. Positive expression was recorded in 15(37.5%) cases of stage II, followed by 13(32.5%) cases in stage I and 8(20%) cases in stage III, while 4(10%) cases showed positive expression in stage IV (Table 2).

The total P21 positive immunohistochemical expression was found in 34(85%) of the cases and as follows; Strong in 16(40%) cases, moderate expression in 12(30%) cases and mild in 6(15%) cases, while no expression was found in 6(15%) cases. The main percentage of positive P21 immunohistochemical expression was located within lower lip in 22(55%) cases followed by alveolus in 7(17.5%) of the cases.

Positive P21immunohistochemical expression was recorded in well differentiated SCC in 17(42.5%) cases. Concerning tumor stage, an equal percentage of positive P21expression shown in stage II & stage I in 12(30%) cases and 7(17.5%) cases in stage III, while 3(7.5%) cases recorded in stage IV, and negative expression was found in 6(15%) cases distributed among I, II and III cases (Table 3).

DISCUSSION

Understanding the molecular basis of OSCC has increased rapidly over the past few years. Knowing more about the pathogenesis of OSCC is essential to improve patient's prognosis and treatment modalities. In this study, the expression of BAD 39(97.5%) of cases indicate that BAD phosphorylation is anti-apoptotic, phosphorelated BAD by Akt forming BAD-protein heterodimer leaving Bcl-2 free to inhibit Bax-triggered apoptosis. Mitochondrial unbounded BAD molecules are then believed to interact with either Bcl-2 or Bcl-X_L and neutralize their anti-apoptotic functions [8].

It has been reported that MDM2 is associated with p53 gene products and may negatively affect the transcriptional activating function of P53. In spite of the absence of P53 assessment in the current study an elevated level of MDM2 expression was found which suggests a P53 independent role for MDM2 in the genesis of malignancies; this finding similar to those of [9,10].

Over expression of MDM2 protein may reflect a persistent response to DNA damaging agents present in OSCC patients and it can be oncogenic independently of P53 via stimulation the S – phase inducing transcription factor E2F1/DP1 or via inhibiting tumor suppressor protein pRB-mediated cell cycle arrest [11]. P21protein mediates cell cycle arrest to secure against DNA replication in cells with anchorage damaged molecules [12].

P21 expression was reported to be correlated with tumor size, grade and stage [13]. Patients displaying loss of p21 had a significantly shorter overall survival rate and poor prognosis [14]. The expression of P21 induces differentiation of normal and transformed cells; it has also been associated with terminal differentiation, senescence and apoptosis.

The immuno-reactivity of P21 in this study was expressed in 34(85%) of the cases of OSCC in areas of squamous differentiation in accord with its function of regulating differentiation of cells, this finding with agreement. The biological significance of the lack P21 expression in tumor cells remains to be elucidated [10].

The P21 gene has a P53 transcriptional regulatory motif and the cells lacking functional P53 express very low level of P21. However, some studies indicate that P53 independent pathways may also lead to increase P21 expression. Expression of P21 is predominant

corresponds to the area of squamous differentiation and also detected in cells with wild type P53 but is often absent in cells lacking P53 activity [15].

Table 1: Clinicopathological finding vs. Immunohistochemical expression of BAD

Clinicopathological parameter N (%)		NE 1(2.5%)	MI 6(15%)	MO 20(50%)	ST 13(32.5%)	Total N (%)
Age Group	0-9 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	40(100%)
	10-19 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
	20-29 2(5%)	0(0%)	0(0%)	1(2.5%)	1(2.5%)	
	30-39 9(22.5%)	0(0%)	3(7.5%)	3(7.5%)	3(7.5%)	
	40-49 5(12.5%)	0(0%)	2(5%)	2(5%)	1(2.5%)	
	50-59 11(27.5%)	0(0%)	1(2.5%)	7(17.5%)	3(7.5%)	
	60-69 6(15%)	1(2.5%)	0(0%)	4(10%)	1(2.5%)	
	70-79 3(7.5%)	0(0%)	0(0%)	0(0%)	3(7.5%)	
	80-89 4(10%)	0(0%)	0(0%)	3(7.5%)	1(2.5%)	
Gender	Male 26(65%)	1(2.5%)	4(10%)	13(32.5%)	8(20%)	40(100%)
	Female 14(35%)	0(0%)	2(5%)	7(17.5%)	5(12.5%)	
Clinical appearance	Ulcer 17(43%)	1(2.5%)	2(5%)	8(20.5%)	6(15%)	40(100%)
	Mass 23(57.5%)	0(0%)	4(10%)	12(30%)	7(17.5%)	
Anatomical Site	Lower lip 24(57.5%)	0(0%)	3(7.5%)	13(30%)	8(20%)	40(100%)
	Cheek 3(7.5%)	0(0%)	0(0%)	2(5%)	1(2.5%)	
	F.O.M 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	
	Alveolus (Mandible) 7(17.5%)	1(2.5%)	1(2.5%)	4(10%)	1(2.5%)	
	Tongue 1(2.5%)	0(0%)	0(0%)	0(0%)	1(2.5%)	
	Soft palate 2(5%)	0(0%)	1(2.5%)	0(0%)	1(2.5%)	
Tumor Grade	WD 18(45%)	1(2.5%)	2(10%)	9(22.5%)	6(15%)	40(100%)
	MD 16(40%)	0(0%)	2(5%)	7(17.5%)	7(17.5%)	
	PD 6(15%)	0(0%)	2(5%)	4(10%)	0(0%)	
Tumor Stage	I 14(35%)	0(0%)	1(2.5%)	6(15%)	7(17.5%)	40(100%)
	II 15(37.5)	1(2.5%)	3(7.5%)	9(22.5%)	2(5%)	
	III 8(20%)	0(0%)	1(2.5%)	4(10%)	3(7.5%)	
	IV 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	

Table 2: Clinicopathological finding vs. Immunohistochemical expression of MDM2

Clinicopathological parameter N (%)		NE 1(2.5%)	MI 5(12.5%)	MO 17(42.5%)	ST 17(42.5%)	Total N (%)
Age Group	0-9 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	40(100%)
	10-19 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
	20-29 2(5%)	0(0%)	0(0%)	1(2.5%)	1(2.5%)	
	30-39 9(22.5%)	0(0%)	1(2.5%)	5(12.5%)	3(7.5%)	
	40-49 5(12.5%)	0(0%)	1(2.5%)	2(5%)	2(5%)	
	50-59 11(27.5%)	0(0%)	1(2.5%)	5(12.5%)	5(12.5%)	
	60-69 6(15%)	0(0%)	1(2.5%)	2(5%)	3(7.5%)	
	70-79 3(7.5%)	1(2.5%)	0(0%)	1(2.5%)	1(2.5%)	
	80-89 4(10%)	0(0%)	1(2.5%)	1(2.5%)	2(5%)	
Gender	Male 26(65%)	1(2.5%)	2(5%)	11(27.5%)	12(30%)	40(100%)
	Female 14(35%)	0(0%)	3(7.5%)	6(15%)	5(7.5%)	
Clinical appearance	Ulcer 18(45%)	0(0%)	1(2.5%)	8(20%)	9(22.5%)	40(100%)
	Mass 22(55%)	1(2.5%)	4(10%)	9(22.5%)	8(20%)	
Anatomical site *	Lower lip 24(60%)	0(0%)	3(7.5%)	11(27.5%)	10(25%)	40(100%)
	Cheek 3(7.5%)	0(0%)	0(0%)	2(5%)	1(2.5%)	
	F.O.M 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	
	Alveolus (Mandible) 7(17.5%)	0(0%)	0(0%)	3(7.5%)	4(10%)	
	Tongue 1(2.5%)	0(0%)	0(0%)	0(0%)	1(2.5%)	
	Soft palate 2(5%)	1(2.5%)	1(2.5%)	0(0%)	0(0%)	
Tumor Grade	WD 18(45%)	0(0%)	0(0%)	11(27.5%)	7(17.5%)	40(100%)
	MD 16(40%)	1(2.5%)	3(7.5%)	3(7.5%)	9(22.5%)	
	PD 6(15%)	0(0%)	2(5%)	3(7.5%)	1(2.5%)	
Tumor stage	I 14(35%)	1(2.5%)	0(0%)	6(15%)	7(17.5%)	40(100%)
	II 15(37.5%)	0(0%)	4(10%)	6(15%)	5(12.5%)	
	III 8(20%)	0(0%)	0(0%)	4(10%)	4(10%)	
	IV 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	

*** (Chi square = 27.59, d.f. =15, p=0.0242)**

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Table 3: Clinicopathological finding vs. Immunohistochemical expression of P21

Clinicopathological parameter N (%)		NE 6(15%)	MI 6(15%)	MO 12(30%)	ST 16(40%)	Total N (%)
Age Group	0-9 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	40(100%)
	10-19 0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
	20-29 2(5%)	0(0%)	0(0%)	1(2.5%)	1(2.5%)	
	30-39 12(30%)	2(5%)	2(5%)	4(105%)	2(5%)	
	40-49 5(10%)	0(0%)	1(2.5%)	2(2.5%)	2(5%)	
	50-59 11(27.5%)	3(7.5%)	1(2.5%)	1(2.5%)	6(15%)	
	60-69 5(12.5%)	1(2.5%)	0(0%)	2(5%)	2(5%)	
	70-79 3(7.5%)	0(0%)	0(0%)	0(0%)	3(7.5%)	
	80-89 4(10%)	0(0%)	2(5%)	2(5%)	0(0%)	
Gender	Male 26(65%)	4(10%)	4(10%)	5(12.5%)	13(32.5%)	40(100%)
	Female 14(35%)	2(5%)	2(5%)	7(17.5%)	3(7.5%)	
Clinical appearance	Ulcer 17(42.5%)	4(10%)	4(10%)	4(10%)	5(12.5%)	40(100%)
	Mass 23(57.5%)	2(5%)	2(5%)	8(20%)	11(27.5%)	
Anatomical site	Lower lip 24(60%)	2(5%)	4(10%)	9(22.5%)	9(22.5%)	40(100%)
	Cheek 3(7.5%)	1(2.5%)	0(0%)	1(2.5%)	1(2.5%)	
	F.O.M 3(7.5%)	2(5%)	0(0%)	0(0%)	1(2.5%)	
	Alveolus (Mandible) 7(17.5%)	0(0%)	2(5%)	1(2.5%)	4(10%)	
	Tongue 1(2.5%)	1(2.5%)	0(0%)	0(0%)	0(0%)	
	Soft palate 2(5%)	0(0%)	0(0%)	1(2.5%)	1(2.5%)	
Tumor Grade *	WD 18(45%)	1(2.5%)	3(7.5%)	5(12.5%)	9(22.5%)	40(100%)
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	PD 6(15%)	0(0%)	3(7.5%)	3(7.5%)	0(0%)	
Tumor stage	I 14(35%)	2(5%)	1(2.5%)	4(10%)	7(17.5%)	40(100%)
	II 15(37.5%)	3(7.5%)	4(10%)	3(7.5%)	5(12.5%)	
	III 8(20%)	1(2.5%)	0(0%)	4(10%)	3(7.5%)	
	IV 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	

***(Chi square = 15.97, d.f. = 6, p= 0.0139)**

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