Immunohistochemical Expressions of AKT, ATM and Cyclin E in Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Understanding the pathogenesis and molecular basis of Oral Squamous Cell Carcinoma (OSCC) has increased rapidly over the past few years that is essential to improve patient's prognosis and treatment modalities. The purpose of this study to evaluate the Immunohistochemical expressions of AKT, ATM, AND Cyclin E in oral squamous cell carcinoma

Materials and methods: This study was performed on a 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 AKT showed positive expression in 38 (95%), ATM showed positive expression in 38(95%) and Cyclin E showed positive expression in 36(90%) of the cases.

Conclusion: A statically significant correlation was found regarding the immunohistochemical expression of AKT with tumor grade and stage, Cyclin E with the age group and ATM with the clinical appearance.

Keywords: Squamous cell carcinoma, oral cancer, immunohistochemistry, prognosis, expression. (J Bagh Coll Dentistry 2016; 28(3):44-51).

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; it occurs in different sites and has many aetiological factors, it is occurring predominantly in alcohol and tobacco-using adults in the 5th and 6th decades of life. 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].

Akt, also known as Protein Kinase B (PKB), is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration [3]. The main biological consequences of Akt activation that are relevant to cancer cell growth can be classified loosely into three categories: survival, proliferation, and growth. Activation of the Akt pathway in cancer cells leads to epithelial–mesenchymal transition and invasion in vivo. Akt induced epithelial–mesenchymal transition involves down regulation of E-cadherin, which appears to result from upregulation of the transcription repressor SNAIL.

Akt has additional effects on tumour induced angiogenesis that are mediated, in part, through hypoxia inducible factor 1α and vascular endothelial growth factor (VEGF) [4]. Akt could promote growth factor-mediated cell survival both directly and indirectly [3].

Ataxia telangiectasia mutated (ATM) is a serine/threonine protein kinase that is recruited and activated by DNA double-strand breaks. It phosphorylates several key proteins that initiate activation of the DNA damage checkpoint, leading to cell cycle arrest, DNA repair or apoptosis. Several of these targets, including p53, CHK2 and H2AX are tumor suppressors. The protein is named for the disorder Ataxia telangiectasia caused by mutations of ATM [5]. The cell cycle has different DNA damage checkpoints, which inhibit the next or maintain the current cell cycle step. There are two main checkpoints, the G1/S and the G2/M, during the cell cycle, which preserve correct progression. ATM plays a role in cell cycle delay after DNA damage, especially after double-strand breaks (DSBs) [6].

Cyclin E is a member of the cyclin family. Cyclin E binds to G1 phase Cdk2, which is required for the transition from G1 to S phase of the cell cycle that determines cell division. The Cyclin E/CDK2 complex phosphorylates p27Kip1 (an inhibitor of Cyclin D), tagging it for degradation, thus promoting expression of Cyclin A, allowing progression to S phase. Like all cyclin family members, cyclin E forms a complex with cyclin-dependent kinase (CDK2). Cyclin E/CDK2 regulates multiple cellular processes by

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phosphorylating numerous downstream proteins [7].

Cyclin E/CDK2 plays a critical role in the G1 phase and in the G1-S phase transition. Cyclin E/CDK2 phosphorylates retinoblastoma protein (Rb) to promote G1 progression. Hyper-phosphorylated Rb will no longer interact with E2F transcriptional factor, thus release it to promote expression of genes that drive cells to S phase through G1 phase. Several mechanisms lead to the deregulated expression of cyclin E. In most cases, gene amplification causes the overexpression [8]. The purpose of this study is to evaluate the Immunohistochemical expressions of AKT, ATM, AND Cyclin E in oral squamous cell carcinoma.

MATERIALS AND METHODS

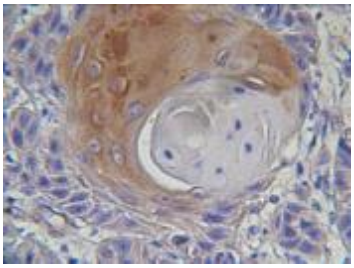
This study was performed on a forty formalin-fixed paraffin-embedded blocks of OSCC cases. All were collected from the Histopathological Laboratory at Maxillofacial Surgery Department at Ramadi Teaching Hospital.

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 were 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.

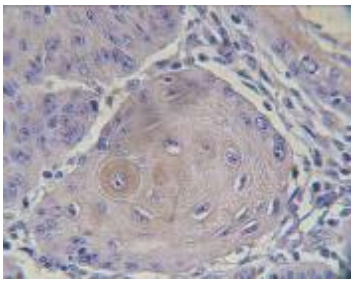
Three sections 5µm for positive and negative tissue and technical control were taken and mounted on positively charged microscopic slides (Biocare medical USA and Afco brand China) to obtain a greater tissue adherence. H & E staining was used for reassessment of histopathological examination of the collected samples and control group. For each specific antibody (AKT, ATM, and Cyclin E, Abcam-USA), the recommended dilution was applied (1/100, 1/100, and 1/100 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 a brown cytoplasmic and /or nuclear staining pattern (according to type of expression for each marker).

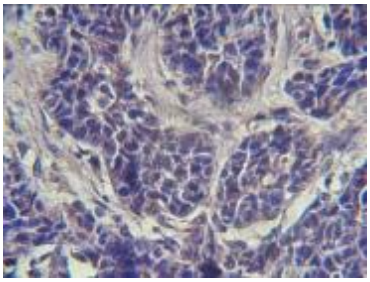
Labeling index for each field was calculated using the following equation: (number of positive cells/ number of total cells); the mean value of labeling indices for the five fields was considered to be the label index for the case. The scoring was done under light microscope and assigned to four categories: **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 was applied for statistical assessment of clinicopathological and immunohistochemical findings to identify the significant or non-significant correlation between them at 95% confidence interval (0.05 level of significance).



A. Positive cytoplasmic expression in WD (40X)

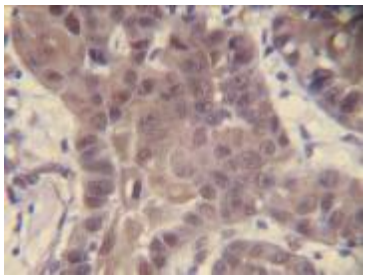


B. Positive cytoplasmic expression in MD (40X)

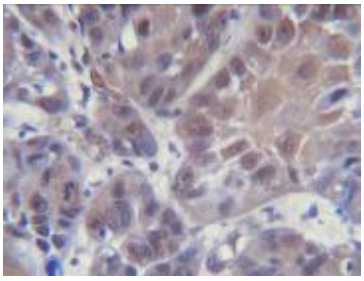


C. Positive cytoplasmic expression in PD (40X)

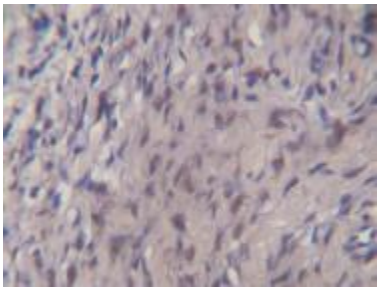
Figure 1 (A, B and C): Immunohistochemical pattern of expression of AKT



A. Positive cytoplasmic expression in WD (40X)

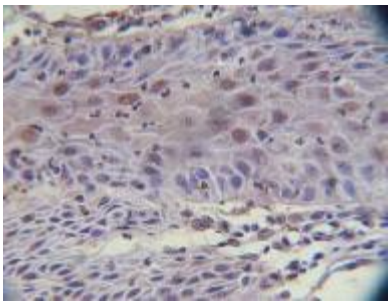


B. Positive cytoplasmic expression in MD (40X)

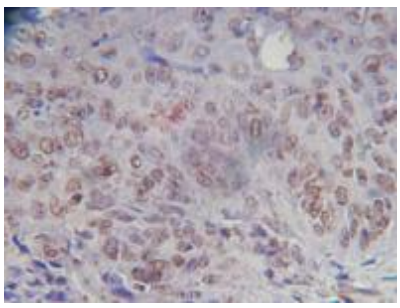


C. Positive cytoplasmic expression in PD (40X)

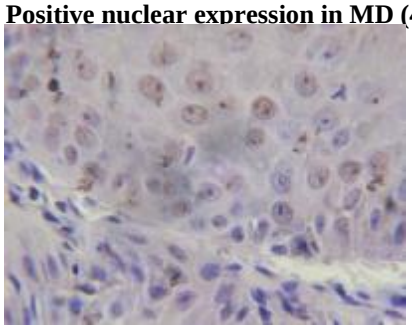
Figure 2 (A, B and C): Immunohistochemical pattern of expression of ATM



A. Positive nuclear expression in WD (40X)



B. Positive nuclear expression in MD (40X)



C. Positive nuclear expression in PD (40X)

Figure 3 (A, B and C): Immunohistochemical pattern of expression of Cyclin E

RESULTS

Forty cases of OSCC were included in this 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 immunohistochemical expression of AKT found in 38 (95%) of the collective cases and as followed; Strong in 21 (52.5%) cases, moderate expression in 15(37.5%) cases and low expression in 2 (5%) cases, while no expression was found in 2 (5%) of the cases. Concerning the anatomical site and according to the number of the cases included in the study, the recorded percentage of immunohistochemical expression of AKT was found in lower lip as a positive expression in 23 (57.5%) cases.

The higher percentage of immunohistochemical expression of AKT was positive expression in well differentiated SCC as seen in 18 (45%) cases, followed by positive moderately differentiated SCC as seen in 16 (40%) case, the higher percentage was shown as a positive expression under stage II in 14 (35%) cases and 14(35%) cases in stage I, followed by 8(20%) cases in stage III, while 2 (5%) cases showed positive expression in stage IV (Table 1).

The total Cyclin E positive immunohistochemical expression was found in 36 (90%) of cases as follow; Strong in 14 cases (35%), moderate expression in 13 cases (32.5%) and low expression in 9 (22.5%) cases. The highest immunohistochemical expression of Cyclin E was located within lower lip as a positive expression in 23(57.5%) cases followed by positive expression in alveolus 5(15%) of the cases. The higher percentage of immunohistochemical expression of Cyclin E was positive expression in well differentiated SCC as seen in 17(42.5%) cases, followed by

positive moderately differentiated SCC as seen in 14(35%) case and a positive expression under stage II in 15 (37.5%) cases, followed by 14 (35%) cases in stage I (Table 2).

The total positive immunohistochemical expression of ATM was found in 38 (95%) of the collective cases and as follow; including Strong expression in 13 (32.5%) cases, moderate expression in 15 (37.5%) of the cases and low expression in 10 (25%) of the cases while negative expression was found in 2 (5%) of the cases the higher percentage of immunohistochemical expression of ATM was shown mainly within lower lip as a positive expression in 24 (60%) cases followed by positive expression in alveolus 6(15%) of the cases and a positive expression in well differentiated SCC as seen in 18 (45%) cases. Concerning the tumor staging, the higher percentage was shown as a positive expression under stage II in 14 (35%) cases and 14 (35%) cases in stage I (Table 3).

DISCUSSION

Tumor or growth in oral region may be divided into benign (noncancerous) and malignant (cancerous). A malignant is life threatening, few patient with malignant tumors that are treatable. Knowing more about the pathogenesis of OSCC is essential to improve patient's prognosis and treatment modalities [(9)]. Cancer research is obtained toward understanding the carcinogenic mechanism by determination of expression and protein immunostaining in oral cancer in comparison with adjacent normal epithelium; highlighting correlation between expression and tumor differentiation. An intensive search for tumor markers based on the molecular alterations in cancerous lesions can be used for efficient diagnosis, prognosis and potential therapeutic targets for different type of human cancer [(1)]. It has been found that in normal cells cyclin E was rapidly down regulated when the cells entered S-phase. However, in the tumour derived cell cultures, the levels of cyclin E instead increased in early S-phase and remained high throughout S and sometimes even G2. Increased cyclin E levels during S and G2 might affect the tumour cells by causing genomic instability, which is a hallmark of cancer. Another interpretation of the rising levels of cyclin E in S-phase in tumour cells is that tumour cells might initiate DNA replication prematurely, with a low level of cyclin E, and thereby cyclin E begins to accumulate first after the cells have entered S-phase. The relatively low levels of cyclin E in tumour cells in G1 support this interpretation [(10)].

In this study we found that cyclin E shows a high expression 38(95%) in all OSCC lesions and showed increased expression with degree of differentiation from poor to well differentiated; this finding is agreed with the results of Zhou et al [(11)]. High expression of cyclin E may play an important role in early stage of carcinogenesis and could be a potential targeted marker to early interfere with cancer progress and stratify high risk patients with precancerous lesion for close surveillance [(12)]. The main biological consequences of Akt activation that are relevant to cancer cell growth can be classified loosely into three categories: survival, proliferation (increased cell number), and growth (increased cell size) [(4)]. Akt pathway is the major survival pathway in cancer cells, which is frequently upregulated in human tumors [(13)].

In this study we found that AKT shows a high expression in positive immunohistochemical staining in 37 (92.5%) of the collective cases of OSCC lesions similar to the finding of Schlieman et al [(24)] showed that p-Akt expression correlated with higher histological tumour grade in pancreatic cancers and Dhawan et al [(14)] who reported higher incidence of p-Akt expression in melanomas. ATM plays an essential role in the pathways activated by DNA breaks [(15)]. In reaction to various agents that damage DNA, ATM phosphorylates p53. Various reports have shown the association of ATM mutation with risk of different human malignancies. The immunoreactivity of ATM in this study was expressed in 38 (95%) of the cases of OSCC, this finding with agreement [(16)]. The findings of elevated level of ATM expression in cases of OSCC suggested ATM kinase plays a critical role in the DNA damage response and its phosphorylation cascade to inhibit the p53-MDM2 interaction, which releases p53 to induce p21 and G1 cell-cycle arrest. overexpressed in OSCCs and might be associated closely with OSCC progression by preventing cell-cycle arrest and apoptosis. Although ATM mainly nucleus localized but we observed that there is a cytoplasmic expression and it is directly proportion with degree of differentiation, this finding in agreement with Xiaolan et al [(17)].

Table 1: Clinicopathological finding vs. Immunohistochemical expression of AKT

Clinicopathological parameter N (%)		NE 2 (5%)	MI 2 (5%)	MO 15(37.5%)	ST 21(52.5%)	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%)	2(5%)	1(2.5%)	6(15%)	
	40-49 5(12.5%)	1(2.5%)	0(0%)	3(7.5%)	1(2.5%)	
	50-59 11(27.5%)	0(0%)	0(0%)	6(15%)	5(12.5%)	
	60-69 6(15%)	0(0%)	0(0%)	3(7.5%)	3(7.5%)	
	70-79 3(7.5%)	0(0%)	0(0%)	0(0%)	3(7.5%)	
	80-89 4(10%)	1(2.5%)	0(0%)	1(2.5%)	2(5%)	
Gender	Male 25(62.5%)	1(2.5%)	1(2.5%)	8(20%)	15(37.5%)	40(100%)
	Female 15(37.5%)	1(2.5%)	1(2.5%)	7(17.5%)	6(15%)	
Clinical appearance	Ulcer 17(42.5%)	1(2.5%)	1(2.5%)	6(15%)	9(22.5%)	40(100%)
	Mass 23(57.5%)	1(2.5%)	1(2.5%)	9(22.5%)	12(30%)	
Anatomical site	Lower lip 24(60%)	1(2.5%)	2(5%)	7(17.5%)	14(35%)	40(100%)
	Cheek 3(7.5%)	0(0%)	0(0%)	3(7.5%)	0(0%)	
	F.O.M 3(7.5%)	0(0%)	0(0%)	1(2.5%)	2(5%)	
	Alveolus (Mandible) 7(17.5%)	1(2.5%)	0(0%)	3(7.5%)	3(7.5%)	
	Tongue 1(2.5%)	0(0%)	0(0%)	0(0%)	1(2.5%)	
	Soft palate 2(5%)	0(0%)	0(0%)	1(2.5%)	1(2.5%)	
Tumor grade *	WD 18(45%)	0(0%)	1(2.5%)	8(20%)	9(22.5%)	40(100%)
	MD 16(40%)	0(0%)	1(2.5%)	4(10%)	11(27.5%)	
	PD 6(15%)	2(5%)	0(0%)	3(7.5%)	1(2.5%)	
Tumor stage *	I 14(30%)	0(0%)	0(0%)	2(5%)	12(30%)	40(100%)
	II 15(37.5%)	1(2.5%)	2(5%)	9(22.5%)	3(7.5%)	
	III 8(20%)	0(0%)	0(0%)	4(10%)	4(10%)	
	IV 3(7.5%)	1(2.5%)	0(0%)	0(0%)	2(5%)	

*** (Chi square = 15.13; df:6; P=0.0192), * (Chi square = 20.85; df:9; P=0.0133)**

Table 2: Clinicopathological finding vs. Immunohistochemical expression of ATM

Clinicopathological parameter N (%)		NE 2(5%)	MI 10(25%)	MO 15(37.5%)	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%)	1(2.5%)	1(2.5%)	3(7.5%)	0(0%)	
	50-59 11(27.5%)	1(2.5%)	1(2.5%)	5(12.5%)	4(10%)	
	60-69 6(15%)	0(0%)	3(7.5%)	1(2.5%)	2(5%)	
	70-79 3(7.5%)	0(0%)	1(2.5%)	1(2.5%)	1(2.5%)	
	80-89 4(10%)	0(0%)	1(2.5%)	1(2.5%)	2(5%)	
Gender	Male 26(65%)	0(0%)	7(17.5%)	11(27.5%)	8(10%)	40(100 %)
	Female 14(35%)	2(5%)	3(7.5%)	4(10%)	5(12.5%)	
Clinical appearance *	Ulcer 17(42.5%)	0(0%)	8(20%)	4(10%)	5(12.5%)	40(100%)
	Mass 23(57.5%)	2(5%)	2(5%)	11(42.5%)	8(20%)	
Anatomical site	Lower lip 24(60%)	0(0%)	6(15%)	10(25%)	8(20%)	40(100%)
	Cheek 3(7.5%)	0(0%)	0(0%)	1(2.5%)	2(5%)	
	F.O.M 3(7.5%)	1(2.5%)	0(0%)	0(0%)	2(5%)	
	Alveolus (Mandible) 7(17.5%)	1(2.5%)	4(10%)	2(5%)	0(0%)	
	Tongue 1(2.5%)	0(0%)	0(0%)	0(0%)	1(2.5%)	
	Soft palate 2(5%)	0(0%)	0(0%)	2(5%)	0(0%)	
Tumor Grade	WD 18(45%)	0(0%)	5(12.5%)	7(17.5%)	6(15%)	40(100%)
	MD 16(40%)	1(2.5%)	3(7.5%)	6(15%)	6(15%)	
	PD 6(15%)	1(2.5%)	2(5%)	2(5%)	1(2.5%)	
Tumor stage	I 14(35%)	0(0%)	4(10%)	4(10%)	6(15%)	40(100%)
	II 15(37.5%)	1(2.5%)	4(10%)	8(20%)	2(5%)	
	III 8(20%)	1(2.5%)	0(0%)	2(5%)	5(12.5%)	
	IV 3(7.5%)	0(0%)	2(5%)	1(2.5%)	0(0%)	

*** (Chi square = 8.81; df:3; P=0.031)**

Table 3: Clinicopathological finding vs. Immunohistochemical expression of Cyclin E

Clinicopathological parameter n(%)		NE 4(10%)	MI 9(22.5%)	MO 13(32.5%)	ST 14(35%)	Total n(%)
Age Group *	0-9 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%)	2(5%)	3(7.5%)	4(10%)	
	40-49 5(12.5%)	2(5%)	2(5%)	0(0%)	1(2.5%)	
	50-59 11(27.5%)	2(5%)	2(5%)	2(5%)	5(12.5%)	
	60-69 6(15%)	0(0%)	1(2.5%)	3(7.5%)	2(5%)	
	70-79 3(7.5%)	0(0%)	1(2.5%)	2(5%)	0(0%)	
	80-89 4(10%)	0(0%)	1(2.5%)	2(5%)	1(2.5%)	
Gender	Male 26(65%)	3(7.5%)	5(12.5%)	8(20%)	10(25%)	40(100%)
	Female 14(35%)	1(2.5%)	4(10%)	5(12.5%)	4(10%)	
Clinical appearance	Ulcer 17(42.5%)	0(0%)	3(7.5%)	8(20%)	6(15%)	40(100%)
	Mass 23(57.5%)	4(10%)	6(15%)	5(12.5%)	8(20%)	
Anatomical site	Lower lip 24(60%)	1(2.5%)	5(7.5%)	11(27.5%)	7(17.5%)	40(100%)
	Cheek 3(7.5%)	0(0%)	1(2.5%)	0(0%)	2(5%)	
	F.O.M 3(7.5%)	1(2.5%)	1(2.5%)	0(0%)	1(2.5%)	
	Alveolus (Mandible) 7(17.5%)	2(5%)	1(2.5%)	1(2.5%)	3(7.5%)	
	Tongue 1(2.5%)	0(0%)	0(0%)	0(0%)	1(2.5%)	
	Soft palate 2(5%)	0(0%)	1(2.5%)	1(2.5%)	0(0%)	
Tumor Grade	WD 18(45%)	1(2.5%)	3(7.5%)	8(20%)	6(15%)	40(100%)
	MD 16(40%)	2(5%)	5(7.5%)	3(7.5%)	6(15%)	
	PD 6(15%)	1(2.5%)	1(2.5%)	2(5%)	2(5%)	
Tumor stage	I 14(35%)	1(2.5%)	0(0%)	6(15%)	7(17.5%)	40(100%)
	II 15(37.5%)	1(2.5%)	6(15%)	5(12.5%)	3(7.5%)	
	III 8(20%)	1(2.5%)	2(5%)	2(5%)	3(7.5%)	
	IV 3(7.5%)	1(2.5%)	1(2.5%)	0(0%)	1(2.5%)	

*** (Chi square = 28.17; df: 15; P=0.0205)**

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