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THE EXPRESSION OF P53 AND MDM4 IN ORAL, LARYNGEAL AND CUTANEOUS SQUAMOUS CELL CARCINOMA; A COMPARATIVE STUDY BY TISSUE MICROARRAY

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Abstract

Background: P53 and MDM4 had been known to have dual mechanism depending on their localization. Nuclear p53 can bind to DNA and activate proapoptotic gene expression; cytoplasmic p53 can trigger transcription-independent apoptosis by directly interacting with Bcl-2 family members.

Objectives: The aim of current study to evaluate and compare the expression of P53, MDM4 in oral , laryngeal and cutaneous SCC by microarray(TMA) and to investigate the correlation of expression of these markers with histopathological grading of tumor.

Methods: One-hundred twenty paraffin embedded SCC sample of Iraqi patients collected during period 2009-2012, retrieved for TMA construction and the expression of P53 and MDM4 was examined by immunohistochemistry.

Results: Data showed males with SCC were more than females (63.3%/76 , 36.7%/44). Most cancer types expressed both nuclear and cytoplasmic staining of P53 and MDM4 in different percentage, cutaneous Scc Nuclear/cytoplasm expression of P53 were (38.8%/94.6%), laryngeal (55%/90%), oral (36.8%/86.8%). Cutaneous SCC N/Cyt expression of MDM4 were (91.8%/72.9%), laryngeal (91.3%/76.3%), oral (97.5%/62.5%).There is no significant difference in expression of both protein markers and tumor types. Obvious significant correlation showed between tumor grading P53 nuclear expression.

Conclusions: P53 and MDM4 were frequently overexpressed in SCC cases and there was a significant correlation between these markers. Nuclear p53 and cytoplasmic MDM4 overexpression can be considered as prognostic factor with tumour grading. High percentage MDM4 overexpression should be considered in their treatment.

Keywords: P53, MDM4, squamous cell carcinoma

Introduction

Cancer is considered as one of the main leading causes of death at the global level. Statistics indicated that 58 million deaths recorded worldwide in 2005 among which 13% were due to cancer (American Cancer Society, 2013).

Studies on oral cancer have indicated that it participates in about 3% of all cancers in the United States and ranked as the eighth most common cancer in males and the fifteenth most malignancy in females. In the worldwide level, oral cancer comes as the eleventh most common cancer. It is worth mentioning that the risk of intraoral cancer is increased as the age increases especially for males (Neville et al, 2009).

In their study, Al-Talabani and Al-Rawi (2002) reported that oral cancer in Iraq is more prevalent and oral squamous cell carcinoma (SCC) was found to account for approximately 4.5% of all cancer cases according to Iraqi cancer registry, and it represents about 91.5% of all oral cancer and 37% of head and neck cancer.

It has been estimated on worldwide level that laryngeal carcinoma was found to predict more than 160,000 new cases of laryngeal cancer in males and 22,000 cases among females in 2005. Such estimations were accounted for approximately 1.7% of all new cancer cases around the world. Furthermore, it has also been reported that most patients were in their fifth decade of life or beyond. It has also been reported that Carcinoma of the larynx to accounts for more than 90% of squamous cell type. Clinical picture of patients varied from localized disease alone in 60% of patients; 25% present with local disease and regional nodal metastatic disease; and 15% present with advanced disease, distant metastases, or both. It has also been pointed to distant metastases to frequently occur mainly in the lungs and liver (Rosai and Ackerman, 2004).

Several studies have targeted cutaneous SCC because it is known to be the second most common form of malignant skin tumors. These studies showed a rising incidence with age and gender so that men are more commonly affected which may due to greater head and neck exposure to ultraviolet radiation (UVR) (Johnson et al, 1992; Foo et al., 2007; Massari et al, 2007). It has been interestingly found that early identification and

treatment by surgical excision in most cases enhances patients 5-year overall survival is 95% with approximately 4% risk of metastasis (Rowe et al., 1992).

It has been shown that about 50% of human cancers have involved mutations of p53 in either inactivating mutations of p53 or deactivating the p53 pathway by increasing its inhibitors and reducing its activators or inactivating its downstream targets. Most of the remaining malignancies work to deactivate P53 which has nuclear function and is best characterized as a transcription factor that binds to specific DNA sequences and transactivates a number of genes with a variety of functions including cell cycle arrest, apoptosis, senescence, in addition to inhibition of angiogenesis as part of the tumor suppressor function of p53 (Teodoro, Evans, Green, 2007). In a relatively recent study, it has been shown that p53 also affects the metabolic switch which occurs in many tumors (Gottlieb, Vousden, 2010). Furthermore, p53 has been shown to exhibit other biological activities that are cytosolic and transcription-independent. Activation of p53 induces apoptosis even in the absence of a nucleus. The role of p53 has been confirmed in vivo studies in which the restoration of p53 function shows marked regression of already established tumors (Martins, Brown-Swigart, Evan, 2006; Xue et al., 2007).

A study by Barboza et al (2008) points to the role of interactions of MDM2-related protein and MDM4 which has emerged as a key negative regulator of p53. It has been demonstrated that MDM4 directly binds to the p53 transactivation domain and inhibits its transcriptional activity and thus contributes to tumor formation. In their study, Francesca Mancini and Fabiola Moretti (2009) showed that the biological activities of MDM4 depend on both transcriptional activity and transcription independent mitochondrial functions. It has also been shown that the main cytoplasmic localization of MDM4 might also imply a regulation of p53-mitochondrial function. Other studies indicated that overexpression of MDM4 was associated with not only tumor progression but also poor prognosis (Yasmine et al., 2007; Prodosmo et al., 2008).

Considering the incidence of oral, laryngeal and cutaneous squamous cell carcinoma in the world especially in developing countries including Iraq, also due to absence of any report in the literature regarding the association between expression of p53/MDM4 with clinicopathological characteristics of these cancers, herein we aimed for the first time, to investigate the staining patterns and clinical significance of p53 and its related gene MDM4 in a well-characterized series of oral, laryngeal and cutaneous SCC specimens using tissue microarray (TMA) technique. The correlation of expression of mentioned markers with histopathological grading of tumor was also examined.

Materials and Methods

The study samples were collected during period 2009-2012 from archival histopathology of Ghazi al Hariry Specialize Hospital of Surgery/ Ministry of Iraqi health; Teaching Hospital College of Dentistry/University of Baghdad. Formalin-fixed, paraffin embedded (FFPE) tumour samples were collected for 120 patients and retrieved for TMA construction in Oncopathology Research Center, Iran University of Medical Sciences, Iran, Tehran.

Tissue Microarray (TMA) Construction

Tissue microarray blocks were constructed as previously described (Mehrazma et al., 2008). Five-µm H&E slides were used to identify and mark out representative areas of tumor tissue. From each corresponding paraffin-embedded block, the representative tumor regions were selected. Microarray samples with a diameter of 0.6 mm were punched from selected regions of each "donor" block and precisely arrayed into a new recipient paraffin block using Tissue Arrayer Minicore (ALPHELYS, Plaisir France). The TMA blocks were constructed in three copies, each containing samples from a different region of each tumor; the mean scoring of three cores was then calculated as the final score.

Immunohistochemistry

Immunohistochemical staining was performed as described previously (Monireh et al., 2013) by using primary antibodies; monoclonal rabbit P53 and monoclonal mouse antibody MDM4(both from Abcam UK). Briefly, after deparaffinization endogenous peroxidase activity was inhibited by hydrogen peroxide 3% for 15 minutes. Antigens were retrieved by autoclaving for 10 minutes in citrate buffer (pH 6.0), and then incubated with primary antibodies with an optimal dilution of 1/100 for p53 and 1/200 for MDM4. The tissues were incubated in EnVision^{TM}/HRP, Dual link Rabbit/Mouse (DAKO, Denmark) and diaminobenzidine (DAB) (DAKO, Denmark) as secondary antibody for 30 minutes at room temperature. Antigen were visualized with addition of 3,3-diaminobenzidine(DAB, DAKO, Denmark) as chromogen to achieve visualization of antigen. Finally TMA sections were counterstained with hematoxylin (Dako), dehydrated in alcohol, clear in xelene (Dako) and mounted for visualization. Negative control, consisting of TBS instead of primary antibody, confirmed the specificity of the staining. Human skin cancer was used as positive control for P53marker and human lung cancer for MDM4 marker.

Evaluation of immunostaining

The immunostained tissue arrays were evaluated using a semi-quantitative scoring system (by SK) after a series was observed on a multi-headed microscope by two other observers (SK and ZM) blind to the clinical and pathological parameters of patients. In difficult cases, the scoring was confirmed by two observers and a consensus was achieved. Initially, TMA slides were scanned at 10×magnification to obtain a general impression of the overall distribution of the tumor cells and positive cells were then assessed semi-quantitatively at higher magnifications and final scores were given. The degree of staining in tumor microarray scores was quantified using a grading system based on positive percentage for both markers: score was graded as: <5 (negative staining), 1 (5-29% positive cells), 2 (30-59% positive cells), 3 (60-100% positive cells).

Statistical Analysis

All data were analyzed using the SPSS statistical software package version 20 (SPSS, Chicago IL, USA). Percentage, median, inter-quartile value and mean rank tests were used to analyze the significance of correlation between P53, MDM4 expression and clinicopathological parameters. A p-value of ≤ 0.05 was considered to be statistically significant.

Results 1-Clinicopathological data

One hundred and twenty paraffin embedded tissue samples of oral, laryngeal and cutaneous SCC were collected from Iraqi teaching hospitals. The expression pattern of p53 and MDM4 were analyzed in three groups of tumor. Of this collection in MDM4 analysis two sample of laryngeal SCC and three sample in cutaneous SCC, and in p53 analysis two sample in oral SCC and three sample in cutaneous were excluded from study due to technical problem in tissue processing or absence of tumor cell within the core.

Table (1) shows the total numbers of studied samples distributed to the grading, well differentiated SCC were 60(50%), moderate differentiated SCC 42(35%) and poor differentiated SCC 18(15%), the females were 44(36.7) and males 76(63.3%) from total number of samples.

Table 1: General characteristics of patients

	Study group					
	Cutaneous SCC		Laryngeal SCC		Oral SCC	
	N	%	N	%	N	%
Tumor Grade						
Well differentiated	25	62.5	12	30.0	23	57.5
Moderately differentiated	10	25.0	20	50.0	12	30.0
Poorly differentiated	5	12.5	8	20.0	5	12.5
Total	40	100.0	40	100.0	40	100.0
Gender						
Female	14	35.0	14	35.0	16	40.0
Male	26	65.0	26	65.0	24	60.0
Total	40	100.0	40	100.0	40	100.0

2-Evaluation of IHC results

The positivity rate of the selected immunostaining of P53 and MDM4 is shown in Fig (1-4). Most samples in table (2) revealed nuclear; cytoplasmic or mostly both nuclear and cytoplasmic positivity.

Assessment of cytoplasmic expression of P53&MDM4

Positive stain was demonstrated in 104 (90.5%) samples; while 11 samples (9.5%) were negatively stained for P53. Samples included 35/37 cutaneous SCC, 36/40 laryngeal SCC and 34/38 oral SCC. Eighty one samples (70.5%) showed positive staining and 34 (29.5%) were negative for MDM4 consisting of 27/37 cutaneous SCC, 9/38 laryngeal SCC and 25/40 oral SCC.

Assessment of nuclear expression of P53 & MDM4

Fifty samples (43.2%) showed nuclear expression of p53, while 65 samples (56.8%) were negative to P53 14/37. Samples included cutaneous SCC, 22/38 laryngeal SCC and 14/40 oral SCC. One hundred and ten (95.6%) of tumors showed nuclear reactivity and five samples were negative for MDM4 (4.4%), including 34/37 cutaneous SCC, 37/38 laryngeal SCC and 39/40 oral SCC.

The cytoplasmic, nuclear expression of P53 and cytoplasmic expression of MDM4 in three groups of cancer showed no significant relationship ($P > 0.05$). While nuclear expression of MDM4 was significantly related to tumor groups studied (0.042) as shown in table 3.

Table 2 : The Scores of cell reactivity of 3 types SCC.

	Study group					
	Cutaneous SCC		Laryngeal SCC		Oral SCC	
	N	%	N	%	N	%
P53_cytoplasmic expression						
Negative (<5%)	2	5.4	4	10.0	5	13.2
Score-I (5-29%)	9	24.3	11	27.5	5	13.2
Score-II (30-59%)	19	51.4	19	47.5	19	50.0
Score-III (60-100%)	7	18.9	6	15.0	9	23.7
Total	37	100.0	40	100.0	38	100.0
P53_nuclear expression						
Negative (<5%)	23	62.2	18	45.0	24	63.2
Score-I (5-29%)	11	29.7	17	42.5	14	36.8
Score-II (30-59%)	3	8.1	5	12.5	0	0.0
Score-III (60-100%)	0	0.0	0	0.0	0	0.0
Total	37	100.0	40	100.0	38	100.0
MDM4_cytoplasmic expression						
Negative (<5%)	10	27.0	9	23.7	15	37.5
Score-I (5-29%)	17	45.9	18	47.4	16	40.0
Score-II (30-59%)	9	24.3	10	26.3	6	15.0
Score-III (60-100%)	1	2.7	1	2.6	3	7.5
Total	37	100.0	38	100.0	40	100.0
MDM4_nuclear expression						
Negative (<5%)	3	8.1	1	2.6	1	2.5
Score-I (5-29%)	9	24.3	7	18.4	6	15.0
Score-II (30-59%)	15	40.5	16	42.1	14	35.0
Score-III (60-100%)	10	27.0	14	36.8	19	47.5
Total	37	100.0	38	100.0	40	100.0

Table 3: The difference in mean of selected tumor markers between the 3 types of SCC.

	Study group			
	Cutaneous SCC	**Laryngeal SCC**	**Oral SCC**	**P**
P53_cytoplasmic expression				0.59[NS]
Range	(0 - 85)	(0 - 85)	(0 - 80)	
Median	33.3	37.5	43.3	
Inter-quartile range	(26.6 - 50)	(20 - 50)	(26.6 - 56.6)	
N	37	40	38	
Mean rank	57.7	54.3	62.1	
P53_nuclear expression				0.09
Range	(0 - 45)	(0 - 56.6)	(0 - 26.6)	
Median	0	3.3	0	
Inter-quartile range	(0 - 6.6)	(0 - 12.5)	(0 - 6.6)	
N	37	40	38	
Mean rank	54.3	66.5	52.7	
MDM4_cytoplasmic expression				0.52
Range	(0 - 70)	(0 - 60)	(0 - 90)	
Median	10	15	8.3	
Inter-quartile range	(3.3 - 30)	(5 - 30)	(0 - 26.6)	
N	37	38	40	
Mean rank	58.3	62.2	53.7	
MDM4_nuclear expression				0.042
Range	(3.3 - 75)	(0 - 90)	(3.3 - 90)	
Median	40	50	55	
Inter-quartile range	(25 - 61.6)	(30 - 70)	(38.3 - 72.5)	
N	37	38	40	
Mean rank	47.8	58.5	67	

To study the simultaneous effect of tumor grade and tumor group (laryngeal, oral and cutaneous SCC) on cytoplasmic and nuclear expression of P53 tumor marker, a multiple linear regression model was used. As shown in table 4, neither tumor grade nor tumor group had an obvious or statistically significant association with mean cytoplasmic expression. The model was not statistically significant and had no explanatory power (determination coefficient less than 0.01). On the other hand, tumor grade had a statistically significant positive effect on nuclear expression of P53 after adjusting for the effect of tumor group. Having a poorly or moderately differentiated tumor increased nuclear P53 expression by 10.5 and 5.2

compared to well differentiated tumors respectively. The model was statistically significant and able to explain 14% of observed variation in the dependent variable (nuclear P53 expression).

Table 2: Multiple linear regression model with P53 expression as the dependent (outcome) variable and tumor grade and type of SCC as the explanatory (independent) variables.

	cytoplasmic expression		**nuclear expression**	
	Partial regression coefficient	**P**	**Partial regression coefficient**	**P**
(Constant)	36.7	<0.001	-1.4	0.64[NS]
Tumor Grade				
Moderately differentiated compared to well differentiated	1.4	0.66[NS]	5.2	<0.001
Poorly differentiated compared to well differentiated	2.7	0.66[NS]	10.5	<0.001
Tumor group				
Laryngeal SSC compared to Cutaneous SSC	-2.8	0.61[NS]	1.6	0.55[NS]
Oral SSC compared to Cutaneous SSC	2.3	0.67[NS]	-2.940	0.27[NS]
	P (Model) = 0.80[NS] **R^2 = 0.009**		**P (Model) = 0.001** **R^2 = 0.14**	

To study the simultaneous effect of tumor grade and tumor group (laryngeal, oral and cutaneous SSC) on cytoplasmic and nuclear expression of MDM4 tumor marker, a multiple linear regression model was used. As shown in table 5, tumor group had no obvious or statistically significant association with mean cytoplasmic MDM4 expression. Tumor grade had a statistically significant positive effect on cytoplasmic expression of MDM4 after adjusting for the effect of tumor group. Having a poorly or moderately differentiated tumor increased cytoplasmic MDM4 expression by 9.5% and 4.7% compared to well differentiated tumors respectively. The model was not statistically significant and had a low explanatory power (determination coefficient =0.04). On the other hand, tumor grade had an obvious (although not significant statistically) positive effect on nuclear expression of MDM4 after adjusting for the effect of tumor group. Having a poorly or moderately differentiated tumor increased nuclear MDM4 expression by 8.6 and 4.3% compared to well differentiated tumors respectively. Oral SSC was associated with a statistically significant increase in MDM4 nuclear expression by 13.8%, while laryngeal SSC only marginally increased the expression by 6% compared to Cutaneous SSC after adjusting (controlling) for the effect of tumor grading. The model was statistically significant and

able to explain 7% of observed variation in the dependent variable (nuclear MDM4 expression).

Table 3: Multiple linear regression model with MDM4 expression as the dependent (outcome) variable and tumor grade and type of SCC as the explanatory (independent) variables.

	cytoplasmic expression		**nuclear expression**	
	Partial regression coefficient	**P**	**Partial regression coefficient**	**P**
(Constant)	9.8	0.041	33.0	<0.001
Tumor Grade				
Moderately differentiated compared to well differentiated	4.7	0.049	4.3	0.17[NS]
Poorly differentiated compared to well differentiated	9.5	0.049	8.6	0.17[NS]
Tumor group				
Laryngeal SSC compared to Cutaneous SSC	0.1	0.99[NS]	6.0	0.28[NS]
Oral SSC compared to Cutaneous SSC	0.2	0.97[NS]	13.8	0.012
	P (Model) = 0.25[NS] **R^2 = 0.04**		**P (Model) = 0.04** **R^2 = 0.07**	

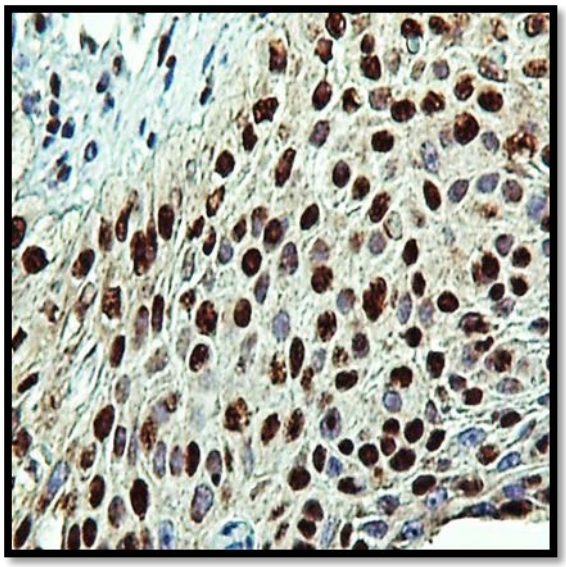
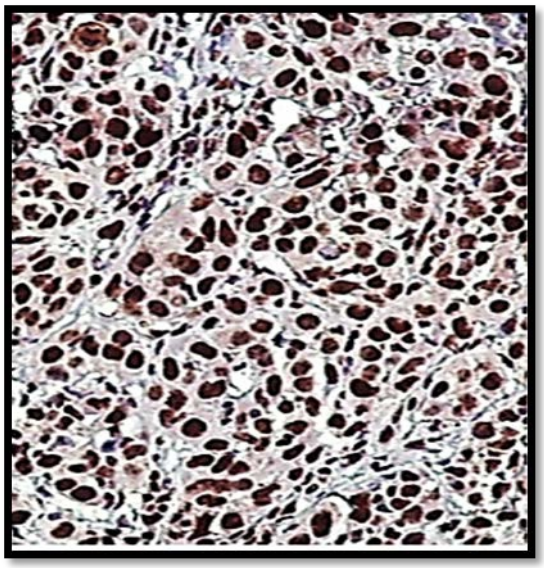
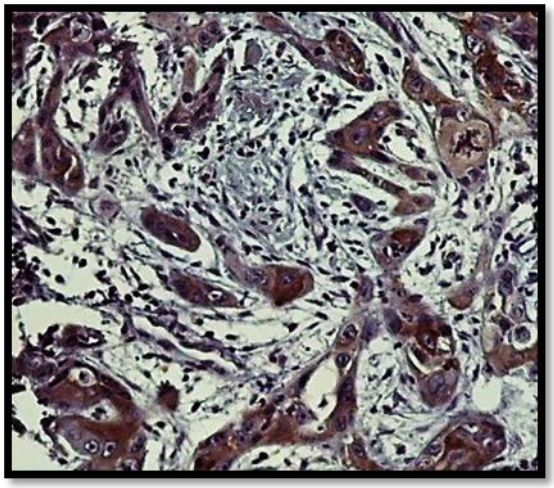


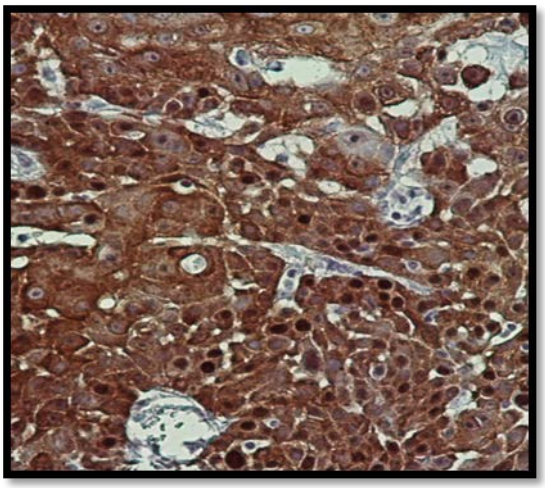
***Fig.(1) Nuclear MDM4 staining of SCC* x200**



Fig(2) Nuclear &cytoplasmic MDM4 staining of SCC. x200



Fig(3) Cytoplasmic P53 stainingof Sccx200



Fig(4)Nuclear &cytoplasmic staining of P53x200

Discussion

Clinicopathological findings 1

The present study showed that about 64% of SCC occurred in males. The findings of the present study are consistent with other studies in Iraq as the study by Al-Talabani (1998) who reported that males were affected more than females. Our findings also agree with other international studies (Axell et al., 1996; Shiboski, 2005).

However, in oral SCC the disparity in the male to female ratio has become less pronounced over the past half century, probably because women have been more equally exposing themselves to known oral carcinogens such as tobacco and alcohol (Neville et al., 2002a). It has been shown that in cutaneous SCC, the males are more likely to be affected more than females due to more outdoor work by males compared with females (William et al, 2011). Approximately most studies concerning laryngeal SCC showed more predominance in males (Sadiq, 2010). According to Cancer Research UK (2013), around 1,900 men and 400 women were diagnosed with laryngeal cancer in the UK in 2010.

Regarding histopathological grading the present study showed that 50% of cases were well differentiated SCC. These results do not agree with other studies in which moderate differentiated were more prevalent (Sadiq, 2010). However other studies showed high frequency of poorly differentiated OSCC (Klijanienko et al., 1995; Janot et al, 1996).

Immunohistochemistry Findings 2-

According to O'Brate and Giannakakou (2003), the function of p53 depends on its localization and nuclear import and export of p53 is a tightly

regulated process. Nuclear localization is required for p53-mediated transcriptional regulation. P53 contains three nuclear localization signals (NLS) that upon stimulation enable its nuclear import whereas nuclear export of p53 to the cytoplasm is mediated by two nuclear export signals (NES). Furthermore, MDM4 represents an important protein for study expression due to its role in the p53 pathway.

A-Nuclear expression

P53 is synthesized in the cytoplasm and must be transported into the nucleus to exert its transcriptional effect. In normal cells under non-stressed conditions, P53 is a short-lived protein which shuttles between the nucleus and the cytoplasm in a cell-cycle specific manner (Hayon and Haupt, 2002) and is maintained in a latent form. In response to stress, however, this shuttle is biased towards nuclear accumulation, which is essential for p53 to elicit its biological effects. In present study nuclear p53 positive was 43.2% without significant difference with tumor groups (cutaneous 37.8%, laryngeal 55%, and oral 36.8%).The study confirmed the extend of many studies that proven the role of mutant p53 protein in carcinogenesis. The overexpression of p53 was shown to be in about half of HNSC studied 63% (35/56) of tumors (Yasmine et al., 2007) and 49% (35/71) of laryngeal SCC showed overexpressed of p53 (Osman et al., 2002).

MDM4 is mainly a cytoplasmic protein (Migliorini et al., 2002). MDM4 is considered as a key regulator of p53 with biological activities that depend on both transcriptional activity and transcription independent mitochondrial functions. MDM4 has the ability to bind p53 and to block its transcriptional activity; however, the main cytoplasmic localization of MDM4 might also imply a regulation of p53-mitochondrial function (Mancini and Moretti, 2009).

The overexpression of MDM4 in retinoblastomas was reported by Laurie et al (2006), and 50% in HNSC (Yasmine et al., 2007). In this study the nuclear overexpression of MDM4 was seen in 95.6% and there was no significant difference with tumor groups (cutaneous 91.8%, laryngeal 97.3%, and oral 97.5%). Other studies have demonstrated increased levels of MDM4 in 40% of head and neck squamous carcinomas (Valentin-Vega et al, 2007) and MDM4 overexpression in about 65% of melanomas, which can lead to the conclusion that MDM4 overexpression is an important oncogenic event that alters p53 function in melanoma in a large proportion of patients. It is also possible that high levels of MDM4 suggest that these inhibitors may substitute for mutations in p53 and therefore, contribute to the severity and progression of the disease. The presence of high levels of MDM4 in samples studied suggests that these data should be considered in the treatment (Valentin-Vega et al, 2007).

2- Cytoplasm expression

In our study, cytoplasmic expression of P53 was 90.5% and there was no significant differentiation between tumor groups (cutaneous 94.6%, laryngeal 90%, oral 86.9%). Sequestered p53 was present in 37% of inflammatory breast carcinomas and greater than 95% of undifferentiated neuroblastomas (NB) but never in differentiated benign derivatives of NB (Moll et al, 1992). Cytoplasmic p53 relocalizes to mitochondria only in response to apoptotic stimuli (Marchenko et al., 2007). In this regard, it is worth to mention that in addition to p53 transcriptional role, stress-induced p53 translocation to mitochondria which induces mitochondrial outer membrane permeabilization is a common early component in p53-mediated apoptosis in normal and transformed cells (Moll et al., 2006). Other studies have also pointed to the fact that under a variety of cell-death-inducing conditions, p53 rapidly moves to the mitochondria; these conditions included experimentally irradiation and ischemic damage of mice in which it has been shown that p53 induces mitochondrial outer membrane permeabilization (MOMP), thereby triggering the release of pro-apoptotic factors from the mitochondrial intermembrane space (Douglas and Guido, 2009).

In present study, cytoplasmic MDM4 overexpressed in 70.5%, there was no significant association with tumor groups (cutaneous 72.9%, laryngeal 76.3%, oral 62.5%). More than half of cases associated with cytoplasmic p53. These results highly suggested that there was functional correlation. The cytochrome C release mediated by p53 depends on its ability to bind members of the antiapoptotic Bcl2 family (i.e BCL2 and Bcl-xL) and to inactivate their inhibitory effect on the proapoptotic proteins Bax and Bak (Mihara et al., 2003; Deng et al., 2006). Francesca et al (2009) analyzed the expression of BCL2, p53 and MDM4 immunocomplex by Wb. They showed that there was an association between p53 and BCL2 which was clearly evident in the presence of MDM4, but not in its absence suggesting that MDM4 greatly enhances this binding.

In the present study, a few cases showed cytoplasmic p53 only and others showed only cytoplasmic MDM4 expression. We suggested that p53 and MDM4 bind another regulating protein which is MDM2, one of the drawbacks of the current study which did not study the expression of MDM2. MDM2 is primarily localized in the nucleus of the cell during non-stressed conditions, but contains both nuclear localization and nuclear export sequences, which allow MDM2 to shuttle between the cytoplasm and the nucleus, MDM2 can actually export active p53 from the nucleus, where it can transactivate genes, to the cytoplasm and no longer interact with DNA. Although outside of the nucleus p53 cannot induce transcription, increasing evidence shows that p53 also performs different activities in the cytoplasm. MDM2 is able to inhibit p53 activity in two major ways. Firstly, it can bind

to the transactivation domain of p53, thereby inhibiting its ability to cause transcription of its targets and secondly, by acting as an E3 ubiquitin ligase of p53, ultimately leading to changes in localization and proteasomal degradation (Miriam ett al., 2012).

Finally, regarding tumor grading and p53 and MDM4 expression as either nucleous or cytoplasmic, there was no obvious statistical significance between proteins expression (cytoplasmic p53, nucleous MDM4) and the degree of malignancy except in p53 localized to nucleus expression and expression of cytoplasmic MDM4 which suggested poor prognosis. Poeta et al (2007) reported on a multicenter prospective analysis of p53 status and survival data for 420 cases of surgically treated HNSCC that p53 mutations being significantly associated with a shorter overall survival in HNSCC compared with wild-type cases. Girod et al (1993) reported that 47% of specimens of well differentiated squamous cell carcinoma, 60% specimens of moderate to poorly differentiated squamous cell carcinoma and 66% specimens of recurrent squamous cell carcinoma were P53 positive. The authors have suggested that mutation of p53 gene is related to increasing dysplasia and loss of differentiation in the carcinogenesis of the oral mucosa. Raybaud et al (1996) reported that most of the published data on oral, head and neck, esophageal and laryngeal squamous cell carcinoma have shown no positive relationship between p53 expression and histological grading of tumors, they concluded that the role played by p53 mutations in the progression and vital prognosis of head and neck squamous cell carcinoma has not yet been demonstrated.

Conclusion

Normally, the level of p53 protein is tightly regulated and not detectable by immunohistochemistry. The overexpression of P53 and MDM4 indicated tumourgensis ability of these proteins. The function of p53 and MDM4 depends on either nuclear or cytoplasmic localization. There was no correlation between proteins expression and tumour grading except nucleus p53 and cytoplasmic MDM4 which indicated a worse prognostic of malignancy. There was no significant difference between tumor groups and both proteins expression. The high expression level of Mdm4 represents promising target for cancer therapy.

Conflict of interest: The authors declare that they have no conflict of interest.

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