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Immunohistochemical Detection of P53 and Bcl-2 in Esophageal Tumors

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ABSTRACT

This study aimed to detect the expression of P53 and Bcl-2 proteins in esophageal tumors using immunohistochemical method. 50 formalin fixed paraffin blocks (FFPB) previously diagnosed as esophageal tumors (40 of them were malignant tumors and 10 were benign tumors). FFPB were cut then stained using immunohistochemical method for the detection of P53 and Bcl2. The age of study samples ranged between 8 and 85 years with mean age of 60 years. The study population included 32 (64%) male and 18 (36%) females. Out of the 40 malignant samples, 34 samples were squamous cell carcinoma and 6 were adenocarcinoma. The immunohistochemical expression of P53 was detected in 23 (46%) sample, (all of them were malignant), and negative in 27 (54%) samples (17samples (34%) of them were malignant and the remaining 10 samples (20%) were benign) with statistical association between P53 expression and malignant tumors of the esophagus (P.value =0.001). Out of 23 positive samples, 19 (38%) were squamous cell carcinoma, and 4 (8%) were adenocarcinoma with no statistical association between the type of tumor and P53 expression (P.value = 0.622). The expression of p53 was compared with degree of histological differentiation and was detected in 11(22%) samples of moderately differentiated tumors, and 11(22%) samples of poorly differentiated tumors with statistical association with the grade of tumor (P.value =0.002). Immunohistochemical expression of bcl2 was detected in 5 (10%) samples and negative in 45 (90%) samples (35 (70%) of them were malignant and 10 (20%) were benign) with no statistical association between Bcl-2 expression and esophageal tumors (P.value =0.239). Out of 5 samples expressing Bcl-2, 4 (8%) of them were squamous cell carcinoma and only one (2%) was adenocarcinoma with no statistical association between Bcl-2 expression and type of cancer (P.value =0.738). Comparing the expression of bcl-2 and the grade of the tumor the positive result was detected in 2 (4%) samples of moderately differentiated tumors and 3 (6%) samples of poorly differentiated tumors with no relation between the histologic grade of tumor and Bcl-2 expression (P.value =0.857). This study concludes that there is association between P53 expression and malignant tumors of esophagus and with histological differentiation of tumor, with no association with type of tumor. Bcl-2 expression is not associated with both type of tumor and the histological grade of tumor.

INTRODUCTION

Esophageal cancer is cancer arising from the esophagus that runs between the throat and the stomach (Montgomery, 2014), and it is one of the least studied and deadliest cancers worldwide because of its extremely aggressive nature and poor survival rate. It ranks sixth among all cancers in mortality (Yuwei, 2013). There are two main types of esophageal cancer ; squamous cell carcinoma and adenocarcinoma (Feldman *et al*., 2010).

The incidence of the two main types of esophageal cancer varies greatly between different geographical areas (Napier *et al*., 2014). In general, esophageal squamous cell carcinoma (ESCC) is more common in the developing world, and esophageal adenocarcinoma (EAC) is more common in the developed world (Montgomery, 2014),and it is one of the top ten most common cancer sites in Khartoum among all registered cancer cases with available information (N = 6548, 96.7%), cancer of esophagus rate = 5.8 per 100,000 (Intisar *et al*., 2014).

Smoking and alcohol consumption , hot tea drinking, red meat consumption, poor oral health, low intake of fresh fruit and vegetables, and low socioeconomic status have been associated with a higher risk of esophageal squamous cell carcinoma. Barrett's esophagus is clearly recognized as a risk factor for esophageal cancer, and dysplasia remains the only factor useful for identifying patients at increased risk, for the development of esophageal adenocarcinoma in clinical practice (Yuwei, 2013).

For different types of esophageal cancer, the risk increases with age, with a mean age at diagnosis of 67 years (Cummings and Cooper, 2008).

The diagnosis should be made from an endoscopic biopsy with the histology to be classified according to the World Health Organization criteria (Stahl *et al.,* 2013).

Curative treatment options for esophageal cancer include surgical resection, external beam radiotherapy, chemotherapy with cisplatin and 5-FU, or combinations of two or three of these options. The main factors for selecting primary therapy are tumor stage and location, histological type and the medical condition, as well as considerations from patients (Rice *et al*., 2010).

Tumor suppressor p53, encoded by the *p53* gene located at chromosome 17q13.1, is highly associated with a poor prognosis in human cancers (Murata *et al*., 2013). P53 is the most frequently mutated gene in human tumors. Mutations occur in almost every type of tumor and in over 50% of all tumors. P53 mutations are found in ~30%–50% of lung, esophageal, colorectal, head and neck, and ovarian cancers, and in ~5% of leukemia, sarcoma, melanoma, testicular cancer, and cervical cancer (Hollstein *et al*., 1991, Olivier *et al*., 2004).

The Bcl-2 gene is located in chromosome 18q21 (Zavrides *et al*., 2005).The Bcl-2family of proto-oncogenes block apoptosis (Reed, *et al*., 2004). It was found to be increased in reflux esophagitis, non dysplastic Barrett's and low grade dysplastic Barrett's epithelium, but or virtually absent in high grade dysplasia or carcinomas (Chatelain and Flejou, 2003).

There are many reports indicating an inverse relationship between mutant p53 over expression and apoptosis (Masuda *et al.,* 2003). Numerous preclinical and clinical studies have suggested that impact of p53 status on responses to chemotherapy or radiotherapy depends on status of other genes such as Bcl-2 (Skirnisdottir *et al*., 2002). There are evidences referring to the development of a multi-drug resistance phenomenon as a result of over expression of Bcl-2protein (Tamm *et al.,* 2001).

MATERIALS and METHODS

Slides Preparation: Two sections of 5µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for immunohistochemistry which was then taken in thermal coated slides and dried in hot plate oven at 80ºC for one hour

Immunohistochemical stain: Sections were brought to water and retrieved using water bath retrieval technique at 97ºC, then treated with hydrogen peroxide solution for 15 minutes, then washed in phosphate buffer saline (PH 7.4) for 5 minutes, then treated with anti P53 and Bcl2 primary antibodies (for separate sections) for 30 minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for 30 minutes, then rinsed in phosphate buffer saline, then treated with DAB for 7 minutes, then washed in phosphate buffer saline for 5 minutes, then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) 2 minutes for each then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media (Bancroft and Marilyn, 2008).

RESULTS

The sex of study subjects revealed that 32 (64%) of them were males and the remaining 18 (36%) were females (Table 1). The histopathology diagnosis of samples revealed that 40 (80%) of them were malignant and the remaining 10 (20%) were benign (Table 2).

Table 1: Distribution of sex among study population

Sex	Frequency	Percent
Male	32	64%
Female	18	36%
Total	50	100%

Table 2: Distribution of sample among the study population

Sample	Frequency	Percent
Malignant	40	80%
Benign	10	20%
Total	50	100%

Table (3) shows the relation between P53, Bcl-2 expression and histopathological diagnosis. Malignant esophageal tumors revealed positive expression of P53 in 23 (46%) patients and negative expression in 17(34%) patients, while all benign tumors 10 (20%) sample showed negative expression of P53 with significant statistical association (P. Value 0.001). Malignant esophageal tumors revealed positive expression of Bcl-2 in 5 (10%) sample and negative expression in 35 (70 %) sample, while all benign tumors show negative expression of Bcl-2 with insignificant statistical association (P. value 0.239). Table (4) showed the relation between P53, Bcl-2 expression and type of tumor and the grade of tumor Expression of p53 revealed that p53 positive in 23 samples, 19 were diagnosed as ESCC and 4 were diagnosed as AC and negative in 17 samples, 15 of SCC and 2 were AC. The analysis showed that no relationbetween expression of P53 and the type of tumor (p. value 0.622). P53 expression and the grade of the tumor showed that p53 expression was positive in 11 moderately differentiated tumors and 11 poorly differentiated tumors. There was

significant relation between p53 expression and the grade of tumor (p.value 0.002).

Table 3: Relation between P53, Bcl-2 expression and histopathology diagnosis

Histopathology diagnosis	P53		Total	Bcl2		Total
	Positive	Negative		Positive	Negative	
Malignant	23	17	40	5	35	40
Benign	0	10	10	0	10	1
Total	23	27	50	5	45	50
P value	0.001			0.293		

Table 4: Relation between P53, Bcl-2 expression and tumor grade

P53 result	Type of tumor		Total	Grade of tumor			Total
	Squamous cell carcinoma	adenocarcinoma		I			
Positive	19	4	23	0	11	11	22
Negative	15	2	17	1	3	5	9
Total	34	6	40	1	14	16	31
P value	0.622			0.002			
Bcl2 result							
Positive	4	1	5	0	2	3	5
Negative	30	5	35	1	12	13	26
Total	34	6	40	1	14	16	31
P value	0.738			0.857			

Expression of bcl-2 showed that Bcl-2 positive in 5samples, 4 were diagnosed as ESCC and 1 was diagnosed as AC and negative in 35 samples, 30 of SCC and 5 were AC with no relation between expressions of bcl-2and the type of tumor (p. value 0.738). The comparison between Bcl-2 expression and the grade of the tumor it was found that Bcl-2 expression was positive in 2 moderately differentiated tumors and 3 poorly differentiated tumors. There was insignificant relation between Bcl-2 expression and the grade of tumor (p value 0.857).

DISCUSSION

Esophageal cancer is the 8th most common cancer and the 6th most common cause of cancer death in the world (Jemal et *al*., 2011). There are many studies which have shown that there was complex alterations of gene expression underlie the development of different malignant phenotypes of esophageal cancer cells (Zhou *et al*., 2003).

Our study included samples containing 32(64%) male and 18 (36%) female from different age groups ranging between 8-85 years, with mean age 60.7 years. A simillar result was observed by Makoto *et al.,* (1997) their study reported that the mean of age of patient ranging between 42-83 years was 61 years.

Accumulation of mutant p53 protein has been demonstrated in a number of human malignancies and has been shown to be associated with a poor prognosis in patients with breast, gastric, and colorectal carcinomas (Thor *et al*., 1992, Joypaul *et al*., 1994, Kawamura *et al*., 1996)

In our study Immunohistochemical detection of p53 showed there was a significant statistical association between the positive expression of P53 and malignant tumor (p. value =0.001). The p53 protein prevents

cells with DNA damage from dividing, and activates the apoptosis pathway, thereby preventing the propagation of cells with such alterations. Disruption of native p53 function inhibits apoptosis and thereby allows expansion of abnormal cell population our result is similar to results observed by Arbabi *et al.,* (2005) who reported that nuclear p53 expression in the neoplastic epithelial cells of esophagus was observed in 67.6% of tumor samples.

The results showed no statistical association between the p53 expression and the type of tumor (p. value =0.622) and this was contradicted with results obtained by Huang *et al.,* (2014) who reported that the expression of p53 in the ESCC tissue was significantly high (P.value = 0.01).

This study showed that there was significant relation between the P53 positive expression and the poor differentiation of tumor (p value =0.002), similar results has been reported by Huang *et al.,* (2014) which showed that the level of p53 protein expression was found to correlate with the pathological grade (P = 0.001). Also this result is compatible with Hamelin, *et al.* (1994) who reported that prevalence increases significantly with advancing histologic grade of dysplasia.

Bcl-2 is a mitochondrial protein with antiapoptotic activities. Over expression of Bcl-2 causes a decrease in growth rate and sensitivity to cytotoxic drugs (Hong e al 2002). High levels of Bcl-2 expression have been demonstrated in a variety of tumor types which is due to deregulation of Bcl-2 expression by several mechanisms (Chan *et al.*, 2004).

Immunohistochemical detection of bcl-2 showed insignificant association between Bcl-2 expression and malignancy (p.value = 0.239), this is compatible with results observed by Sarbia *et al.,* (1996), in their study in bcl-2 expression in carcinomas of the esophagus only 48 (32.0%) out of 150 showed cytoplasmatic bcl-2 expression Bcl-2 positive samples were compared with the type of tumor and it was found that 4/5 positive samples were diagnosed as esophageal squamous cell carcinoma, and 1/5 was adenocarcinoma. With insignificant relationship between each type of malignancy and the Bcl-2 positive result (p value= 0.738).

Bcl-2 and the grade of the tumor the positive result was not observed in well differentiated while it had been observed in 2 moderately differentiated, 3 poorly differentiated and here it was found an insignificant relation between the grade of tumor and expression of Bcl-2 (p value =0.857), as observed by Sarbia, *et al*., (1996) who reported that Bcl-2 expression was correlated inversely with tumor differentiation, occurring more frequently in G3 and G4 carcinomas (47.1%) than in G1 and G2, also no correlations were found between bcl-2 expression and stage.

On this study the co-expression of the two marker was P53-/ bcl2- 16 (52%), P53+/bcl2 –19 (38%), P53-/bcl2 + 1(2%) , P53+/bcl2 + 4 (8%) it was found that no correlation between the expression of p53 and bcl-2 in esophageal tumor (p value =0.288), and this result is contradicted with the results of Arbabi *et al.,* (2005) in Iran who observed over expression of mutated p53 protein and wild type Bcl-2 in esophageal tumor samples with lower histological differentiation. Co-expression of p53 and Bcl-2 implies that functional alterations in p53 protein may affect transcription regulation of Bcl-2 and consequently the over expression of BcI-2 protein (Blagosklonny, 2001).

CONCLUSION

The study concluded that here is association between P53 expression and malignant tumors of esophagus, and also with histological differentiation of tumor with no association with type of tumor.

Bcl-2 expression is not associated with both type of tumor and the histological grade of tumor.

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