Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Immunohistochemical Detection of HPV Ag in Oesophageal Tumours

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ARTICLE INFO

ARTICLE HISTORY
Received: 8th August 2015
Accepted: 14 October 2015
Available online: June 2016

KEYWORDS:

HPV, Oesophagus adenocarcinoma, Oesophagus squamous cell carcinoma

ABSTRACT

This study aimed to detect the expression of HPV Ag in oesophageal tumours using immunohistochemical method. Sixty formalin fixed paraffin blocks (FFPB) previously diagnosed as oesophageal tumours (50 of them were malignant oesophageal tumours and 10 were benign oesophageal tumours) were used in this study. Blocks were cut and stained by immunohistochemical method (New indirect method) for detection of HPV Ag. The data obtained were analyzed using SPSS program. The age of patients ranged between 30 and 85 years with mean age of 60 years. The study subjects included 33 (55%) males and 27 (45%) females. Out of fifty malignant samples, 41 samples were squamous cell carcinoma and 9 samples were adenocarcinoma. The immunohistochemical expression of HPV Ag was detected in 12 (20%) samples and negative in 48 (80%) samples. 10/12 positive samples were malignant and the remaining 2/12 were benign, with no statistical association between HPV infection and malignant tumours of the oesophagus (P =0.646). The degree of histological differentiation revealed 39 (78%) samples were moderately differentiated tumours, 7 (14%) samples were poorly differentiated tumours and 4(8%) samples were well differentiated tumours with insignificant statistical association with the HPV infection (P>0.05). This study concludes that there is no association between HPV infection and malignant tumours of the oesophagus.

INTRODUCTION

Oesophageal carcinoma is the eighth most common cancer and the sixth most common cause of cancer related deaths worldwide with developing nations making up more than 80% of total cases and deaths (Herszényi *et al*., 2010). The two main sub-types of the disease are oesophageal squamous-cell carcinoma (SCC), which is more common in the developing world, and oesophageal adeno-carcinoma (AC), which is more common in the developed world. A number of less common types also occur. SCC arises from the epithelial cells that line the oesophagus AC arises from glandular cells present in the lower third of the oesophagus, often where they have already transformed to intestinal cell type (Barrett's oesophagus) (Kelsen and David, 2007).

In Sudan, the oesophageal cancer considered as seventh most common cancer sites in Khartoum (Awadelkarim *et al.,* 2012). Risk factors of oesophageal cancer include smoking and alcohol use. AC is thought to almost always arise in the setting of Barrett's oesophagus, which is a condition in which the normal lining of the oesophagus is replaced by lining normally found in the stomach. Other pre-existing oesophageal conditions may also increase the risk of developing SCC of the oesophagus. Conditions like achalasia (Fiorica *et al*., 2004).

The first step is to establish the diagnosis of oesophageal cancer. Initial tests include a barium swallow, where the person swallows barium to permit visualization of the contours of the oesophagus on X-rays. An endoscopy is commonly done when people first present with symptoms. The standard of care includes performing an ultrasound during the endoscopy, called an endoscopic ultrasound examination (Siegel *et al*., 2015).

Treatment is based on the cancer`s stage and location, together with the person's general condition and individual preferences. Small localized squamous-cell cancers may be treated with surgery alone with the hope of a cure. In most other cases, chemotherapy with or without radiation therapy is used along with surgery. Larger tumours may have their growth slowed with chemotherapy and radiation therapy (Stahl *et al.,* 2013).

Human Papilloma Virus (HPV) is a DNA virus from the papillomavirus family that is capable of infecting humans. The role of HPV in the causation of oesophageal tumours is unclear. Many studies examined the association between oesophageal tumours and infection by immunohistochemical studies (Jesper *et al.,* 1998). This study aimed to detect the HPV which may be considered as a risk factor for this tumour.

MATERIALS AND METHODS

Sample collection: Paraffin embedded tissue blocks previously diagnosed as oesophageal tumours were collected for this study from different centres in Khartoum State.

Slide preparation: One section of 5µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for immunohistochemistry which is 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 HPV

primary antibody 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 (new indirect method) (Bancroft and Marilyn, 2008).

Data analysis: The data were analyzed using version 11.5 SPSS computer program; frequencies, means and chi-square correlations were calculated.

RESULTS

The sex of study subjects revealed that 33 (55%) were males and 27 (45%) were females (Table 1). Table (2) shows the age of study subjects, less than 55 years were 24 (40.0%), 5 to 80 years were 32 (53.3%) and 81 to 100 years were 4 (6.7%). Table (3) showed that malignant oesophageal tumours revealed positive expression of HPV in 10 (16.7%) samples and negative expression in 40 (66%) samples, while benign tumours gave positive result in 2 (3.3%) sample and 8 (13.3%) showed negative result with insignificant association (P=>0.05) also showed insignificant relation between HPV infection and the grade of tumour (P>0.05). Well differentiated oesophageal adeno-carcinoma showed positive expression of HPV Ag (Figure 1). Oesophageal squamous cell carcinoma showed positive expression of HPV Ag (Figure 2)

Table 1: Distribution of sex among study population

Sex	Frequency	Percent %
Male	33	55
Female	27	45
Total	60	100

Table 2: Age among the study population

Age group (year)	Frequency	Percent %
Less than 55 years	24	40.0
55_ 80 years	32	53.3
81 _ 100 years	4	6.7
Total	60	100%

Table 3: Relation between immunohistochemical expression of HPV Ag and tumour type

Histopathology diagnosis		HPV expression		Total
		Positive	Negative	
Malignant	Well differentiated tumour	1	3	4
	Moderately differentiated tumour	7	32	39
	Poorly differentiated tumour	2	5	7
Benign		2	8	10
Total		12	48	60
P value			>0.05	

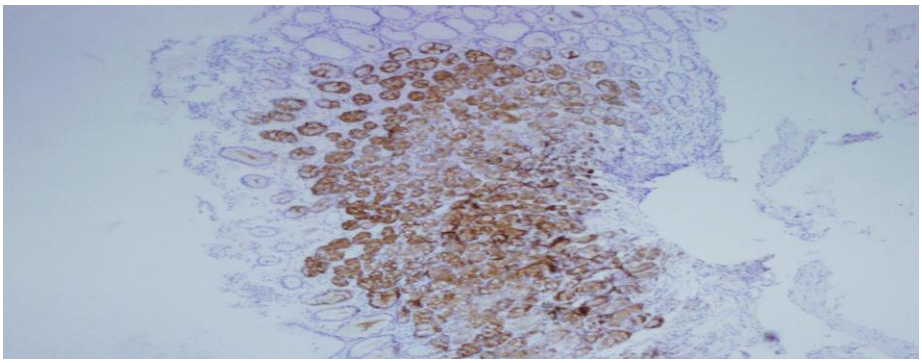

Figure 1: Well differentiated oesophageal adenocarcinoma with positive expression of HPV Ag (10×)

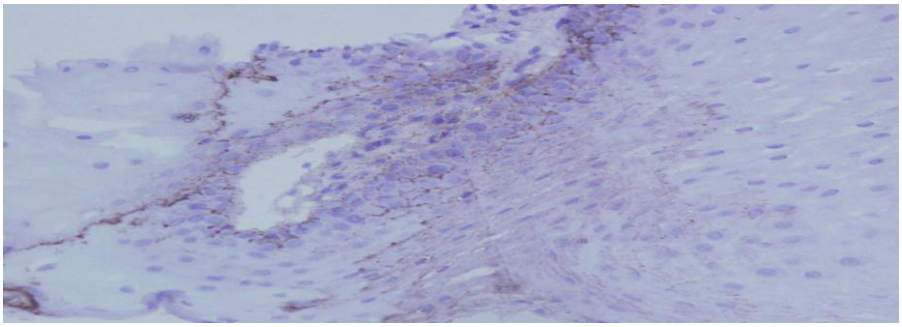

Figure 2: Oesophageal squamous cell carcinoma with positive expression of HPV Ag (40×)

DISCUSSION

Oesophageal cancer (EC) is one of the most common gastrointestinal malignancies with a strong aggression in the developing countries (Jemal *et al*., 2011). HPV has been established as a risk factor for oropharyngeal squamous cell carcinoma and HPV-associated cancers has been associated with an improved prognosis (Ang *et al*., 2010).

Many studies have showed that there is complex alterations of gene expression underlie the development of different malignant phenotypes of oesophageal cancer cells (Zhou *et al*., 2003). The present study included samples containing 33(55%) male and 27(45%) female from different age groups ranging between 30-85 years, with mean age 60 years.

Human papilloma viruses (HPV) consist of a heterogenic group of viruses (32 different HPV types currently recognized) known to induce a variety of squamous cell tumours (papillomas and warts) in the skin, and on mucous membranes of respiratory, gastrointestinal, and genitourinary tracts (Syrjänen,1986).

HPV has been implicated in SCC (16 and 18 types) transforming proteins E6 and E7 which interact with p53 protein and retinoblastoma (Rb) protein respectively, leading to loss of their products. These interactions lead to inactivation of the growth suppressive effects of the p53 and Rb proteins, resulting in abnormal proliferative states (Suk and Kum, 1998). Immunohistochemical detection of HPV Ag showed that 10/50 (20%) of malignant tumour and 2/10 were benign tumours

with insignificant association between HPV infection and malignancy (P>0.05). This finding is consistent with Syrjänen, (2002) result who reported that out of 239 oesophageal squamous cell papillomas, HPV have being detected in 51 (21.3%) cases. Also the study is similar to the study of Fangli *et al*., (2014) who reported that HPV was detected in 29 of the 105 patients (27.6%) with ESCC

HPV Ag positive was not correlated with the types of tumour, 6/10 positive samples were oesophageal squamous cell carcinoma, and 4/10 were adeno-carcinoma with insignificant relationship between each type of malignancy and the HPV Ag positive result (P>0.05). This finding is consistent with the study of Jesper *et al*., (1998), they found that there was no evidence of a positive association between HPV16 or HPV18 infection and either form of oesophageal cancer

Concerning the relation between HPV and the grade of tumour the study found that the positive expression was not related to the type of the grades observed (P=0.784), indicating the aggressiveness of tumour is not initiated by the presence or the absence of the infective agent, with no relation found between the proliferation activity of the tumours .

CONCLUSION

The study concludes that there was expression of HPV Ag found in the tissues affected with oesophageal tumours in both malignant and benign with no correlation with histological differentiation.

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