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IDENTIFICATION OF HUMAN PAPILLOMAVIRUS IN ESOPHAGEAL CANCER IN SUDAN

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ABSTRACT
Background: Esophageal cancer, mostly squamous cell carcinomas, is the eight most common cancers worldwide. There are a number of suspected genetic or environmental etiologies. Human papilloma virus (HPV) is said to be a major etiology in areas with high incidence of esophageal carcinoma. This study was designed to investigate the presence of HPV in esophageal cancer in Sudan**. Methods:** A total of 102 formalin fixed paraffin embedded blocks previously diagnosed as esophageal cancers were tested by immunohistochemistry for HPV 16/18. SPSS version 11.5 computer programs were used to analyze the data, frequencies, and means, the P.value was calculated by Chi square test. **Results:** This analysis included 102 patients with esophageal cancer were included in this study (55% male and 45% female), their age ranging from 21to 98 years with mean age of 59.0 years old, squamous cell carcinoma comprised (89%), while (11%) is adenocarcinoma, HPV was detected in HPV was 13.7 0% without statistically significant differences between squamous and adenocarcinoma**. Conclusions:** HPV infection may be one of many factors contributing to development of esophageal cancer.

KEYWORDS: Human papillomavirus, esophageal cancer, Immunohistochemistry.

INTRODUCTION
Esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer, worldwide.[1,2] The etiology of this cancer remains unclear, the epidemiological studies suggest that tobacco smoking, heavy alcohol drinking, micronutrient deficiency.[3,4] and dietary carcinogen exposure may cause the malignancy. Infectious agents have been implicated, as either direct carcinogens or promoters.

Majority of squamous cell carcinomas of the cervix are caused by HPV types 16 and 18.[5,6] there are strongly associated with cancers of the head and neck, particularly the oropharynx and HPV also.[7,8] The viral oncogenes products E6 and E7 play a key role in HPV-associated carcinogenesis, abrogating p53 and retinoblastoma tumor suppressor functions, respectively.[9,10] E7 binds to and degrades Rb, releasing E2F, leading to p16INK4A overexpression, hereafter denoted as p16, which is associated with superior clinical outcome.[11,12]

In particular, human papillomavirus (HPV) has been postulated as a possible cause of ESCC.[13] HPV infection in esophageal cancer was first suggested in 1982 based on histological observations.[14]Subsequent studies using various methods have confirmed the presence of HPV in ESCC.[13,15]

3. MATERIALS AND METHODS
Clinical samples: This is retrospective descriptive study aimed to detect the HPV (16.18) in esophagus tumor using immunohistochemistry. The group included 102 participants, their age ranging from 21-98 years with mean age of 59.03 years old. 102 paraffin blocks that previously were diagnosed as esophageal cancer (91 were esophageal squamous cell carcinoma and 11 were adenocarcinoma) were selected from Ibn Sina Hospital, Soba teaching hospital, Khartoum teaching hospital and national health laboratory Sudan during the period from May 2012 to November 2014 .Patient identification data

were retrieved from patients records data include age, sex and type of esophageal cancer.

Sample collection and preparation

From each paraffin blocks two sections were cut into 4µm thickness, sections were floated into preheated 40c using water bath; one section was placed in coated slide for immunohistochemistry and the other for Hematoxylin and Eosin.

Immunohistochemistry

Immunohistochemistry (Dako kit) for HPV 16 and 18 E6 early proteins was performed following the manufacturer's instructions. Briefly, paraffin-embedded sections were dewaxed; antigen retrieval was performed by heating the sections for 30 minutes in phosphate buffer saline. Endogenous peroxides activity was blocked with 3% hydrogen peroxidase for 10 minutes, then washed in phosphate buffer (PBS) for 2 minutes. then section was incubated with primary antibodies (HPV) for 30 minutes at room temperature in a moisture chamber, and then rinsed in phosphate buffer saline for 2 minutes. Immune section incubated with primary antibody enhancer for 15 minutes, then washed in phosphate buffer for 2 minutes, then secondary antibody labeled with horse reddish peroxidase was applied for 15 minutes. Sections were incubated in diaminobenzidine tetra hydrochloride to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for 1-3 minutes, and then washed in phosphate buffer for 2 minutes. (Dako kit).

Counter stain: Sections were counter stained with Mayer haematoxylin and blued in running tap water for 5 minutes and dehydrated in ethyl alcohol, then cleared in xylene 2 minutes for each, finally mounted using DPX media. The immuno histochemistry dark-brown signals scattered in the infected tumor cells. Positive and negative controls for HPV are stained parallel with test sections.

RESULT

Patient characteristics

A total of 102 patients previously diagnosed as esophageal cancer (55% (56) males and 45% (46) females), their age ranging from 21to 98 years with mean age of 59.0 years old. The majority of patients is in the fifth and sixth decants as in table 4.1.

As regard to esophageal cancer histological subtypes: squamous cell carcinoma comprised (89%), while (11%) is adenocarcinoma, figure 1.

Analysis of HPV

IHC result revealed that the positive rate of HPV was 13.7 0% without statistically significant differences between HPV infection and the histological subtypes of the cancer as showed in (Table 2 and 3).

Table.1: Description of study population by age

Age group	Frequency	Percent
20-40	12	11.8
41-60	48	47.0
More than 60	42	41.2
Total	101	100

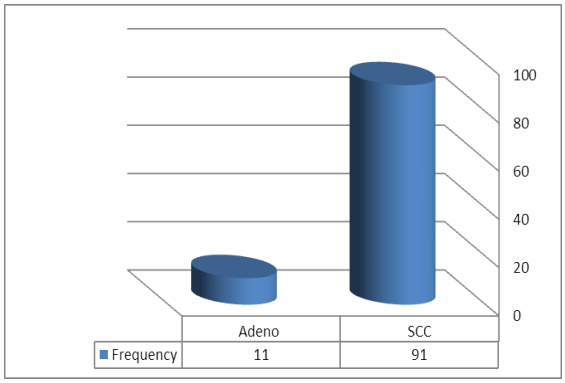

Figure 1.: The frequency of esophageal cancer subtypes

Table. 2: Frequency of HPV immunohistochemical results among study group

HPV	Frequency	Percent
Positive	14	13.7
Negative	88	86.3
Total	102	100

Table.3: Relation between HPV immunohistochemical results and esophagus cancer subtypes.

Esophagus tumor	HPV immunohistochemical result		Total	P.value
	Positive	Negative		
SCC	11	80	91	0.167
Adeno	3	8	11	
Total	14	88	102	

DISCUSSION

Several studies have demonstrated involvement of HPV in benign and malignant lesions of esophagus.[16] Since first report in 1982 by Syrjanen.[17] who found HPV related histological changes in 40% of patients with esophageal carcinoma. Many studies have reported presence of high frequency of HPV DNA in esophageal carcinomatous lesions from China, South Africa and Alaska natives.[16] Also reports from Japan[18,19] and France[20] have also implicated HPV role in esophageal squamous cell carcinoma.

The high-risk HPV types are closely related to malignancies. According to previous studies, HPV-16 is the most prevalent type in squamous cell carcinoma, followed by HPV-18.[21] while other high-risk HPV types are rare[22, 23]. The etiological role of HPV in ESCC is still unclear. The incidence of HPV in ESCC varies between different geographical areas.[17]

The majority of patients is in the fifth and sixth decants, the observation was consistent with United state study by (Enzinger and Mayer) ,they found the risk factor increase with age- most patients are over 60,and median in United state is 67.[24]

Regarding the sex association, the disease is more common in men; these findings were in agreement with the international findings as in 2008 esophageal cancer is 3 to 4 times more common among males than females (Age-Standardized Esophageal Cancer Incidence Rates by Sex and World Area. Source: GLOBOCAN 2008).

The positive rate of HPV infection in esophageal cancer was (14/102) 13.7 0%; suggesting that HPV infection may be an integral part of a multistep process leading to esophageal cancer; a restriction of our study is not to detect the other subtypes except HPV-16/18. The observation was consistent with the previous studies in high-risk areas for ESCC in China.[25,26]

CONCLUSION although there are many accumulating evidence on the presence of the HPV genome in cancer samples, and the malignant transformation of esophageal epithelial cells by the oncogenic HPV types, need furthers investigations to establish the causal role of HPV in esophageal carcinogenesis.

REFERENCES

1. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010; 127(12): 2893–2917.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer Journal for Clinicians. 2011; 61(2): 69–90.
3. Holmes RS, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. Seminars in Radiation Oncology. 2007; 17(1): 2–9.
4. Wei W-Q, Abnet CC, Qiao Y-L, et al. Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. The American Journal of Clinical Nutrition. 2004; 79(1): 80–85.
5. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The New England Journal of Medicine*. 2003; 348(6): 518–527
6. Meschede W, Zumbach K, Braspenning J, et al. Antibodies against early proteins of human papillomaviruses as diagnostic markers for invasive cervical cancer. *Journal of Clinical Microbiology*. 1998; 36(2): 475–480.
7. Herrero R, Castellsagué X, Pawlita M, et al. Human papillomavirus and oral cancer: the international agency for research on cancer multicenter study. *Journal of the National Cancer Institute*. 2003; 95(23): 1772–1783.
8. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *The New England Journal of Medicine*. 2007; 356(19): 1944–1956.
9. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nature Reviews Cancer*. 2002; 2(5): 342–350.
10. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. *Virus Research*. 2002; 89(2): 213–228.
11. Reimers N, Kasper HU, Weissenborn SJ, et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *International Journal of Cancer*. 2007; 120(8): 1731–1738.
12. Kumar B, Cordell KG, Lee JS, et al. EGFR, p16, HPV titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *Journal of Clinical Oncology*. 2008; 26(19): 3128–3137.
13. Syrjänen KJ. HPV infections and oesophageal cancer. Journal of Clinical Pathology. 2002; 55(10): 721–728
14. Syrjänen KJ. Histological changes identical to those of condylomatous lesions found in esophageal squamous cell carcinomas. Archive fur Geschwulstforschung. 1982; 52(4): 283–292
15. Chang F, Syrjänen S, Shen Q, et al. Evaluation of HPV, CMV, HSV and EBV in esophageal squamous cell carcinomas from a high-incidence area of China. Anticancer Research. 2000; 20(5 C): 3935–3940.
16. Chang, F., Syrjanen, S., Wang, L. et al. Infectious agents in the etiology of esophageal cancer. Gastroentrology, 1 992; l03: I 336- 1348.
17. Syrjanen, K.J. Histological changes identical to those ofcondylomatous lesions found in esophageal squamous cell carcinomas. Arch. Geschwulstforsch, 1982; 52: 283-292.
18. Toh, Y., Kuwano, H., Tanaka, S. et al. Detection ofhuman papillomavirus DNA in esophageal carcinoma in Japan by polymerase reaction. Cancer, 1992; 70: 2234-2238.
19. Furihata, M.,Ohtsuki, Y., Ogoshi, S. ct al. Prognostic significance of human papillomavirus genomes (type-16, 18) and aberrant expression ofp53 protein in human esophageal cancer. Int. J. Cancer, 1993; 54:226-230.
20. Benarnouzig, R., Pigot, F., Quiroga, G. et al. Human papillomavirus infection in esophageal squamous cell carcinoma in Western countries. Int. J. Cancer, 1992; 50:549-552.
21. Muñoz N. Human papillomavirus and cancer: the epidemiological evidence. Journal of Clinical Virology. 2000; 19(1-2): 1–5.
22. Moberg M, Gustavsson I, Gyllensten U. Real-time pcr-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. Journal of Clinical Microbiology. 2003; 41(7): 3221–3228.

23. Mork J, Lie AK, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. The New England Journal of Medicine. 2001; 344(15): 1125–1131.
24. Enzinger PC, Mayer RJ. Esophageal cancer.2003: *N Engl J Med*. 349: 2241–2252.
25. Gao Y, Hu N, Han X, Giffen C, Ding T, Goldstein A, Taylor P. Family history of cancer and risk for esophageal and gastric cancer in Shanxi, China. 2009. BMC Cancer. 9: 269.
26. Wen D, Shan B, Wang S, Zhang L, Wei L, Zhou W, Peng Q. A positive family history of esophageal/gastric cardia cancer with gastric cardia adenocarcinoma is associated with a younger age at onset and more likely with another synchronous esophageal/gastric cardia cancer in a Chinese high-risk area. 2010. Eur J Med Genet. 53: 250–255.