

Indexed, Abstracted and Cited: **ISRA Journal Impact Factor, International Impact Factor Services (IIFS), Directory of Research Journals Indexing (DRJI), International Institute of Organized Research and Scientific Indexing Services, Cosmos Science Foundation (South-East Asia), International Innovative Journal Impact Factor, Einstein Institute for Scientific Information {EISI}, Directory of Open Access Scholarly Resources, Science Library Indexing (UAE), Swedish Scientific Publication (Sweden)**

[itefactor.org](http://itefactor.org) **journals indexing**

**Directory Indexing of International Research Journals**

## World Journal of Biology and Medical Sciences

Published by Society for Advancement of Science®

ISSN 2349-0063 (Online/Electronic)

Volume 3, Issue- 1, 1-8, January to March, 2016

Journal Impact Factor: 4.197



WJBMS 3/01/08/2016

All rights reserved

A Double Blind Peer Reviewed Journal / Refereed Journal

[www.sasjournals.com](http://www.sasjournals.com)

[wjbmedsc@gmail.com](mailto:wjbmedsc@gmail.com) / [wjbms.lko@gmail.com](mailto:wjbms.lko@gmail.com)

RESEARCH PAPER

Received: 08/10/2015

Revised: 24/11/2015

Accepted: 30/11/2015

### Detection of Human Papilloma Virus Type 16 and 18 E6 Early Protein among Sudanese Esophageal Tumors Patients using Immuno histochemistry

Mohammed Mohie Eddin Saeed, \*Mohammed Sideeg Abd Elaziz and \*Ibrahim Bakhit Yousif Elemam

Sudan University for Sciences and Technology, Sudan

\*Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Sudan University for Sciences and Technology, Sudan

#### ABSTRACT

*Esophageal cancer is the eight most common cancer worldwide and the sixth cause of cancer related death with squamous cell carcinoma (SCC) accounting for almost half of all esophageal cancers. Oncogenic human papilloma virus (HPV) has been hypothesized as a risk factor for esophageal squamous cell carcinoma. The aims of this review was to estimate the present of (HPV 16 and 18) by immuno histochemical technique In this is descriptive retrospective study, which carried out in Ibn sena Hospital-Khartoum-Sudan, formalin fixed paraffin embedded blocks from 30 patients previously diagnosed as esophageal tumors were included (20 of them with esophageal malignant tumor and 10 of them were benign) ,their age ranging from 8-98 years with mean age of 59 years old.*

**Five (4) micron was taken on positively coated slide for immuno histochemical detection of HPV (16 and 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.**

**The study involved (30) subject twenty out of them were males (66.7%) and (33.3%) were females with (2:1) male: female ratio. Sven samples (23.3%) were positive for HPV (16, 18), while 23(76.7%) were negative, with no statistically significant difference between HPV infection and type of tumors.**

**HPV infection, especially type 16, 18 should be considered as a risk factor for esophageal malignancies in Sudan.**

**Keywords: Human Papilloma virus, Esophageal Cancer and Immuno histochemistry.**

## INTRODUCTION

Esophageal carcinoma (EsC) affects more than 450000 people worldwide and the incidence is rapidly increasing (Pennathur, *et al.*2013). Currently, EsC is the eighth most common incident cancer in the world because of its extremely aggressive nature and poor survival rate (Enzinger and Mayer, 2003; Mao, *et al.*2011).

EsC exhibits an epidemiologic pattern distinct from all other cancers (Blot, 1999; Engel, *et al.*2003). The incidence of esophageal adenocarcinoma has increased sharply over the past few decades, both by period and birth cohort. Etiological studies are required to explain the rapid increase of this lethal cancer (Lepage, *et al.*2008).

The etiologies of squamous cell esophageal carcinoma seem to differ between low-incidence regions and high-incidence regions. For example, tobacco and alcohol use are important risk factors for esophageal cancer in industrial countries (Castellsague, *et al.*2000), whereas in high-incidence regions other factors, such as nutritional deficiency, have a stronger relation to disease incidence (Sharp, *et al.*2001; Sammon.2007). Northern China is one of the world's highest incidence regions (Li.1982; Munoz and Buiatti.1996), but studies that have been carried out there have not yet identified the basis for the high incidence (Xibib, *et al.* 2003; Tran, *et al.*2005).

Involvement of human papilloma virus (HPV) infection in esophageal cancer was first suggested in 1982 (Syrjanen.1982), but this studies that have tested this hypothesis have not obtained consistent results (de Villiers, *et al.*1999; Zhang, *et al.*2011).

Esophageal cancer usually occurs as either squamous cell carcinoma in the middle or upper one-third of the esophagus, or as adenocarcinoma in the lower one-third or junction of the esophagus and stomach (Jemal, *et al.*2010; Fritz, *et al.*2000). In the highest risk area, stretching from northern Iran through the central Asian republics to North-Central China, often referred to as the "esophageal cancer belt," 90% of cases are squamous cell carcinomas (Tran, *et al.* 2005; Gholipour, *et al.* 2008).

Major risk factors for squamous cell carcinomas in these areas are not well understood, but are thought to include poor nutritional status, low intake of fruits and vegetables, and drinking beverages at high temperatures (Islami, *et al.* 2009; Wu, *et al.* 2009). In low-risk areas such as the United States and several Western countries, smoking and excessive alcohol consumption account for about 90% of the total cases of squamous cell carcinoma of the esophagus (Engel, *et al.* 2003). Smoking, overweight and obesity, and chronic gastroesophageal reflux disease, which triggers Barrett's esophagus, are thought to be the major risk factors for adenocarcinoma of the esophagus in the United States and some Western countries (Engel, *et al.* 2003; Kamangar, *et al.* 2009).

A number of studies also found smokeless tobacco products and betel liquid (with or without tobacco) as risk factors for esophageal cancer in certain parts of Asia (Lee, *et al.* 2005; Wu, *et al.* 2006).

Infection with human papillomavirus (HPV), especially HPV type 16, has been implicated as a possible risk factor for esophageal cancer in three seroepidemiologic studies (Han, *et al.* 1996; Bjorge, *et al.* 1997). In two prospective studies (Dillner, *et al.* 1995; Bjorge, *et al.* 1997), HPV16 seropositivity was associated with a more than six fold excess risk. Although the number of observed cases did not permit separate analyses by histologic type, the association appeared to be the strongest in the case of squamous cell carcinoma (Dillner, *et al.* 1995; Bjorge, *et al.* 1997).

## MATERIALS AND METHODS

**Clinical samples:** This is retrospective descriptive study aimed to detect the HPV (16.18) in esophagus tumor using immuno histochemistry. The group included 30 participants, their age ranging from 8-98 years with mean age of 59.03 years old. Thirty paraffin blocks that previously were diagnosed as esophageal tumor (20 were esophageal cancer and 10 were benign) were selected from Ibn Sina Hospital- Sudan during the period from May to November 2014. Patient identification data were retrieved from patients records data include age and sex

### 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 immuno histochemistry and the other for Hematoxylin and Eosin.

### Immuno histochemistry

Immuno histochemistry (Quartett 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 peroxidase 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. (Quartett 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

## RESULT

The study involved 30 subject twenty out of them were Samples from 30 patients previously diagnosed as esophageal tumors including (20 with esophagus cancer and 10 were benign), twenty were males (66.7%) and ten were females (33.3%), their age ranging from 8-98 years with mean age of 59.03 years old.

The majority of esophageal tumors patients were above 50 years old 21(70%) and 9 (30%) were less than 50 years old as in (Table .1).

IHC result revealed that the positive rate of HPV was 23.3 0% and in the comparison between the type of tumor and HPV immuno histochemical results, the study revealed that, the positive expression of HPV in the malignant tumor samples was detected in six patients, while only one case express IHC HPV among benign tumor, with no statistically significant differences between the association of HPV infection and the type of tumor as showed in (Table 2 and 3)

**Table. 1. Description of study population by age.**

Age group	Frequency	Percent
≤50	9	30
>50	21	70
Total	30	100

**Table. 2. Frequency of HPV immuno histochemical results among study group.**

HPV	Frequency	Percent
Positive	7	23.3
Negative	23	76.7
Total	30	100

**Table 3. Relation between HPV immune histochemical results and esophagus tumors.**

Esophagus tumor	HPV immuno histochemical result		Total	P. value
	Positive	Negative		
Malignant	6	14	20	0.222
Benign	1	9	10	
Total	7	23	30	

## DICUSSION

There are more than 130 HPV types identified and these have been classified into low- or high-risk groups according to their potential for oncogenesis (Zur, 2009). 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 (Muñoz, .2000), while other high-risk HPV types are rare (Moberg, *et al.* 2003; Mork, *et al.* .2001).

Recently HPV has been established as a risk factor for oropharyngeal squamous cell carcinoma (Ang, *et al.* 2010). The histologic similarities between the oropharyngeal squamous epithelium and upper esophagus may suggest that HPV can infect esophagus along the route. Our study aimed to investigate the association between HPV and the esophageal tumor.

The present study observed that most of esophageal tumor were over 50 years old, 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 (Enzinger and Mayer. 2003).

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).

In our study, we observed an association between HPV infection and esophageal tumor, HPV was detected in 23.3% of the cases by the use of immuno histochemistry; 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 (Gao, *et al.* 2009; Wen, *et al.*, 2010). In our observation HPV infection present in both benign and malignant esophageal tissue without statistically significant difference as the P.value was 0.222

Our results are agree with HPV studies conducted by Poljak ,*et al*, according to the studies reviewed, it is likely that HPV infection plays a much more significant role in esophageal carcinogenesis with a high incidence of ESCC(Poljak ,*et al.*, 1998)

In 2010(Xueqian, *et al*) was found HPV infection is common in esophageal carcinoma independent of region and ethnic group of origin. Findings in the study raise the possibility that HPV is involved in esophageal carcinogenesis.

Another study by Freddy,*et al*, they conclude 'There are statistically significant associations between esophageal squamous cell carcinoma and seropositivity for E6 for the high-risk mucosal type HPV16 and for the low-risk mucosal type HPV6 ,but not for any of the other HPV type '(Freddy,*et al.*, 2012)

## CONCLUSION

The possible mechanisms of HPV associated carcinogenesis in the esophagus have been discussed in more detail recently. (2 Syrjänen and Syrjänen.2000; Syrjänen. *et al* 1996; Syrjänen. 2000) Despite the 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 intensive lobber to establish the causal role of HPV in esophageal carcinogenesis.

## ACKNOWLEDGMENTS

The authors thank Dr. Hajhamad Alzain (Biochemistry Department, Shendi University) for his expert suggestions and technical assistance.

## REFERENCES

- Ang, K.K., Harris, J., Wheeler, R., Weber, R., Rosenthal, D.I., Nguyen-Tân, P.F., Westra, W.H., Chung, C.H., Jordan, R.C. and Lu, C. (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.*; 363:24–35. [PMC free article] [PubMed]
- Bjorge, T., Hakulinen, T., Engeland, A., Jellum, E., Koskela, P., and Lehtinen, M., (1997). A prospective, seroepidemiological study of the role of human papillomavirus in esophageal cancer in Norway. *Cancer Res*; 57:3989-92.
- Blot, W.J., (1999). Invited commentary: more evidence of increased risks of cancer among alcohol drinkers. *Am J Epidemiol.* 150:1138–140.

- Castellsague, X., Munoz, N., De Stefani, E., Victora, C.G., Quintana, M.J., and Castelletto, R., (2000). Smoking and drinking cessation and risk of esophageal cancer (Spain). *Cancer Causes Control* 11:813–8.
- Cummings, L.C. and Cooper, G.S., (2008). Descriptive epidemiology of esophageal carcinoma in the Ohio Cancer Registry. *Cancer Detect Prev.* 32:87.
- De Villiers, E.M., Lavergne, D., Chang, F., Syrjanen, K., Tosi, P. and Cintonino, M., (1999). An interlaboratory study to determine the presence of human papillomavirus DNA in esophageal carcinoma from China. *Int J Cancer* .81:225–8.
- Dillner, J., Knekt, P., Schiller, J.T. and Hakulinen, T., (1995). Prospective seroepidemiological evidence that human papilloma virus type 16 infections is a risk factor for oesophageal squamous cell carcinoma. *BMJ* .311:1346.
- Engel, L.S., Chow, W.H., Vaughan, T.L., Gammon, M.D., Risch, H.A., Stanford, J.L., Schoenberg, J.B., Mayne, S.T., Dubrow, R. and Rotterdam, H., (2003). Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst.* 95:1404–1413.
- Enzinger, P.C. and Mayer, R.J., (2003). Esophageal cancer. *N Engl J Med.* 349:2241–2252.
- Freddy Sitas, Sam Egger, Margaret I. Urban, Philip R. Taylor, Christian C. Abnet, Paolo Boffetta (2012). InterSCOPE Study: Associations Between Esophageal Squamous Cell Carcinoma and Human Papillomavirus Serological Markers. *J Natl Cancer Inst.* Jan 18,; 104(2): 147–158.
- Fritz, A., Percy, C. and Jack, A., (2000). International Classification of Diseases for Oncology. 3<sup>rd</sup> edition. Geneva: World Health Organization.
- Gao, Y., Hu, N., Han, X., Giffen, C., Ding, T., Goldstein, A. and Taylor, P. (2009). Family history of cancer and risk for esophageal and gastric cancer in Shanxi, China. *BMC Cancer*; 9:269. [PMC free article] [PubMed]
- Gholipour, C., Shalchi, R.A. and Abbasi, M., (2008). A histopathological study of esophageal cancer on the western side of the Caspian littoral from 1994 to 2003. *Dis Esophagus*.21: 322-327.
- Han, C., Qiao, G., Hubbert, N.L., Li, L., Sun, C. and Wang, Y. (1996). Serologic association between human papillomavirus type 16 infection and esophageal cancer in Shaanxi Province, China. *J Natl Cancer Inst* .88(20):1467-1471.
- Islami, F., Boffetta, P., Ren, J.S., Pedoeim, L., Khatib, D. and Kamangar, F., (2009). High-temperature beverages and foods and esophageal cancer risk—a systematic review. *Int J Cancer.* 125: 491-524.
- Jemal, A., Center, M.M., Desantis, C. and Ward, E.M., (2010). Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev.*19: 1893-1907.
- Kamangar, F., Chow, W.H., Abnet, C.C. and Dawsey, S.M., (2009). Environmental causes of esophageal cancer. *Gastroenterol Clin North Am.* 38: 27-57.
- Lee, C.H., Lee, J.M. and Wu, D.C., (2005). Independent and combined effects of alcohol intake, tobacco smoking and betel quid chewing on the risk of esophageal cancer in Taiwan. *Int J Cancer.* 113: 475-482.
- Lepage, C., Rachet, B., Jooste, V., Faivre, J. and Coleman, M.P., (2008). Continuing rapid increase in esophageal adenocarcinoma in England and Wales. *Am J Gastroenterol.* 103:2694–2699.
- Li, J.Y., (1982). Epidemiology of esophageal cancer in China. *Natl Cancer Inst Monogr* .62:113–20.

- Mao, W.M., Zheng, W.H. and Ling, Z.Q., (2011).** Epidemiologic risk factors for esophageal cancer development. *Asian Pac J Cancer Prev.*12:2461–2466.
- Moberg, M., Gustavsson, I. and Gyllensten, U. (2003).** Real-time pcr-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. *Journal of Clinical Microbiology.* ;41(7):3221–3228. [PMC free article] [PubMed]
- Mohammad Muzaffar Mir, Javid Ahmad Dar, Nazir Ahmad Dar, A. T. Syed, Irfana Salam, and Ghulam Nabi Lone, (2007).** The Association of Beta-catenin Gene Mutations and Human Papillomavirus in Carcinoma of Esophagus in a High-Risk Population of India. *Int J Health Sci (Qassim).* 1(2): 177–183.
- Mork, J., Lie, A.K., Glatte E, et al. (2001).** Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *The New England Journal of Medicine;* 344 (15): 1125–1131. [PubMed]
- Munoz, N. and Buiatti, E., (1996).** Chemoprevention of oesophageal cancer. *IARC Sci Publ.*27–33.
- Muñoz N. (2000).** Human papilloma virus and cancer: the epidemiological evidence. *Journal of Clinical Virology;* 19(1-2):1–5. [PubMed][New York: Wiley & Sons.
- Pennathur, A., Gibson, M.K., Jobe, B.A. and Luketich, J.D., (2013).** Oesophageal carcinoma. 381:400–412.
- Poljak, M., Cerar, A. and Seme, K, (1998).** Human papillomavirus infection in esophageal carcinomas: a study of 121 lesions using multiple broad-spectrum polymerase chain reactions and literature review. *Human Pathol.* 29 (3):266-71.
- Sammon, A.M., (2007).** Carcinogens and endemic squamous cancer of the oesophagus in Transkei, South Africa. Environmental initiation is the dominant factor; tobacco or other carcinogens of low potency or concentration are sufficient for carcinogenesis in the predisposed mucosa.69:125–31.
- Sur, M. and Cooper, K., (1998).** The role of the human papilloma virus in esophageal cancer. 30(4):348-54.
- Syrjänen, K., Chang, F. and Syrjänen S. (1996).** Infectious agents as etiological factors in esophageal carcinogenesis. In: Tahara E, Sugimachi K, Oohara T. eds. Recent advances in gastroenterological carcinogenesis I. Bologna: Monduzzi Editore, 1996:29–43.
- Syrjänen, K. and Syrjänen, S. (2000).** Papillomavirus infections in human pathology.
- Syrjänen, K. (2000).** HPV infections of the oesophagus. In: Syrjänen K, Syrjänen S. Papillomavirus infections in human pathology. New York: J Wiley & Sons, 2000:413–28.
- Syrjanen, K.J., (1982).** Histological changes identical to those of condylomatous lesions found in esophageal squamous cell carcinomas.52:283–292.
- Tran, G.D., Sun, X.D. and Abnet, C.C., (2005).** Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. *Int J Cancer.* 113: 456-463.
- Wen, D., Shan, B., Wang, S., Zhang, L., Wei, L., Zhou, W. and Peng, Q., (2010).** 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. *Eur J Med Genet;* 53:250–255. [PubMed]

- Wu, I.C., Lu, C.Y. and Kuo, F.C., (2006).** Interaction between cigarette, alcohol and betel nut use on esophageal cancer risk in Taiwan. *Eur J Clin Invest.*; 36: 236-241.
- Wu, M., Liu, A.M. and Kampman, E., (2009).** Green tea drinking, high tea temperature and esophageal cancer in high- and low-risk areas of Jiangsu Province, China: a population-based case-control study. *Int J Cancer.*124: 1907-1913.
- Xibib, S., Meilan, H., Moller, H., Evans, H.S., Dixin, D. and Wenjie, D., (2003).** Risk factors for oesophageal cancer in Linzhou, China: a case-control study. *Asian Pac J Cancer Prev.* 4:119–24.
- Xueqian Wang, Xiuyun Tian, Fangfang Liu, Yiqiang Zhao, Min Sun and Dafang Chen (2010).** Detection of HPV DNA in –esophageal cancer specimens from different regions and ethnic groups: a descriptive study. *BMCCancer.*10: 19.
- Zhang, Q.Y., Zhang, D.H., Shen, Z.Y., Xu, L.Y., Li, E.M., Au, W.W., (2011).** Infection and integration of human papillomavirus in esophageal carcinoma. *Int J Hyg Environ Health.* 214:156–61.
- Zur Hausen H. (2009).** Papillomaviruses in the causation of human cancers—a brief historical account. *Virology*; 384(2):260–265. [PubMed]

---

**Corresponding author: Dr. Ibrahim Bakhit Yousif Elemam, Faculty of Medical laboratory Sciences, Shendi University, Sudan.**

**Email: [elemam.ibrahim@yahoo.com](mailto:elemam.ibrahim@yahoo.com)**