

# Esophageal cancer in the Sudan: focus on the screening for ebv and the surgical management

## Abstract

**Objective:** The aim of this study was to screen for the existence of Epstein Barr Virus (EBV) in esophageal cancer (EC) tissue samples obtained from Sudanese patients and to review the available literature associated with surgical management.

**Methodology:** In this retrospective study 102 formalin fixed paraffin wax embedded tissue's blocks were retrieved from histopathology laboratories in Khartoum City, Sudan and subsequently tested by Immunohistochemical methods for the presence of EBV. All specimens were previously diagnosed as having esophageal carcinoma.

**Results:** As regards Immuno histochemistry (IHC) staining for EBV, positive findings were revealed in 22 (21.5%) and 79/ (78.5%) of the study subjects were negative. Of the 55 males with esophageal cancer, 15/55 (27%) were identified with EBV infection and the remaining 40/55(73%) found without EBV infection. Of the 46 females with esophageal cancer, 7/46(13%) were found positive for EBV immuno staining and the remaining 39/46(87 %) were found negative for EBV.

**Conclusion:** EBV might have some etiological effects contributing to the occurrence of esophageal cancer in Sudan. Crucial preventive strategies are extremely needed to reduce the burden of esophageal cancer in Sudan.

**Keywords:** esophageal cancer, Sudan, EBV, etiological effects, esophageal cancer

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**Abbreviations:** EC, esophageal cancer; IHC, immuno histochemistry; EBV, epstein barr virus; CMV, cytomegalovirus; HSV, herpes simplex virus

## Introduction

Esophageal cancer (EC) occurs commonly, particularly in Asia, and it is the sixth leading cause of cancer deaths worldwide.<sup>1</sup> Esophageal cancer classically happens in one of two forms, squamous cell carcinomas (SCCs) arising from the stratified squamous epithelial lining of the organ, and adenocarcinoma (AC) arising from columnar glandular cells that replace the squamous epithelium.<sup>2</sup> Sarcomas and small cell carcinomas usually constitute less than 1%-2% of all esophageal cancers.<sup>3</sup> The major risk factors for esophageal cancer include; gastro-esophageal reflux,<sup>4</sup> tobacco smoking, alcohol drinking,<sup>5</sup> viruses,<sup>6</sup> susceptibility genes,<sup>7</sup> low levels of intake of fruits and vegetables.<sup>8</sup> The relationship between viral infection and esophageal cancer was previously reported for several viruses including; high human Papillomavirus (HR-HPV) particularly subtype 16, herpes simplex virus 1 (HSV-1), Epstein-Barr virus (EBV) and cytomegalovirus (CMV). However, the mechanisms of the viral action in addition to geographic variation in infection rates of these viruses still need more efforts.<sup>9</sup> EBV is an oncogenic virus that has been described to be linked to Burkitt's lymphoma, Hodgkin's disease, breast cancer, gastric carcinoma, nasopharyngeal carcinoma and leiomyosarcoma.<sup>10</sup> However, whether EBV is associated to esophageal SCC still controversial: there are limited studies and their conclusions vary.<sup>11</sup>

## Surgical management

Although esophageal cancer is the most common gastrointestinal (GI) cancer in the Sudan, but there a deep absence of literature from the Sudan, particularly in regard to surgical management. In a recent study a total of 114 patients were enrolled in the study, with a mean age of 47years SD +/- 19 and a male to female ratio of 1: 1.04. Malignant causes were mainly due to esophageal cancer (40.4%) and cancer of the stomach cardia (3.5%).Therapeutic intervention was attempted in 83% of the cases.<sup>12</sup> A recent published study investigated 100 consecutive patients who experienced esophagectomy in Shaab Hospital in Khartoum during the period June 2003-Aug 2007. The mean age was 55±14years with an equal sex ratio. Fifty five per cent of patients presented with stage III&IV locally advanced and or metastatic disease. Sixty seven percent of the patients underwent a 2-stage resection, Lewis Tanner type while 27% underwent a 3-stage resection, McKeon operation and 6% had total gastrectomy with distal esophagectomy and roux-en-y reconstruction. The 30days postoperative mortality was 10%. In 75patients who could be traced, the overall 5years survival was 21%(n=16) and the 10years survival was 8% (n=6).<sup>13</sup> Therefore, the aim of this study was to screen for the presence of EBV in esophageal cancer tissue samples obtained from Sudanese patients and to review the available literature associated with surgical management.

## Materials and methods

In this study we investigated 102 Sudanese patients with esophageal carcinomas, 55 were males and 46 were females (males'/females' ratio, 1.20:1.00), their ages ranged between 12 and 98years with mean

age of 60years old. These patients with esophageal carcinomas, were examined for the existence of HR-HPV subtypes 16&18 using LMP1 (Latent membrane protein 1 (LMP1) of EBV) Antibody immuno histochemistry. The diagnosis of esophageal cancer was established depending on clinical examination and histopathological features of the biopsy. EC diagnosis was confirmed base on Royal College of Pathologists criteria (Royal College of Pathologists, 2005). The esophageal cancer including 91/102(89.2%) squamous cell carcinomas (SCCs) and 11/102(10.8%) adenocarcinoma. The sample included full coverage of patients with esophageal lesions referred to Radiation and isotope Centre Khartoum with available sample quantity and data. Ethical consent was approved from ethical committee of the Faculty Research Board and Hospital. LMP1 immuno histochemistry (IHC) was performed on formalin-fixed paraffin embedded (FFPE) tissue sections using kits from (Dako Agilent Technologies Company). Following antigen retrieval using Citrate buffer (pH 6) for 30minutes, sections were incubated with mouse monoclonal LMP1, and then Envision System HRP anti-mouse was employed, followed by diaminobenzidine (DAB) chromogenthen counterstained with Harris' Hematoxylin. Known LMP1-positive sections were used as positive controls, and sections without addition of primary antibody were used as negative controls. All LMP1 IHC tissue sections were semi quantitatively evaluated by two examiners for concentration of staining in the cytoplasm and nucleus of the cells. Intensity of the staining was counted as 0 (none), +(weak), ++(moderate), or +++(strong), with 0 or+scores regarded as negative and ++ or +++considered as positive. LMP1 scoring was done without knowledge of EBV infection status.

**Results**

The present study retrieved 102 tissue blocks prepared from esophageal carcinoma's specimens (91patients were with squamous cell carcinoma and 11 were with adenocarcinoma). Of the 102 study subjects 55 were males and 46 were females their age ranging from 12 to 98years old with a mean age of 60years. The majority of the study populations were at the age range 66-75 years constituting 26 patients followed by age ranges, both 56-65 and <45years representing 24patients. Moreover, 16 patients were identified among age group 46-55years, hence only 12patients were found among age group 76+years, as indicated in Figure 1.



**Figure 1** Description of the study population by age.

Regarding IHC staining for EBV, positive findings were revealed in 22(21.5%) and 79/(78.5%) of the study subjects were negative, as shown in Microphotographs 1 & 2. Of the 55 males with esophageal

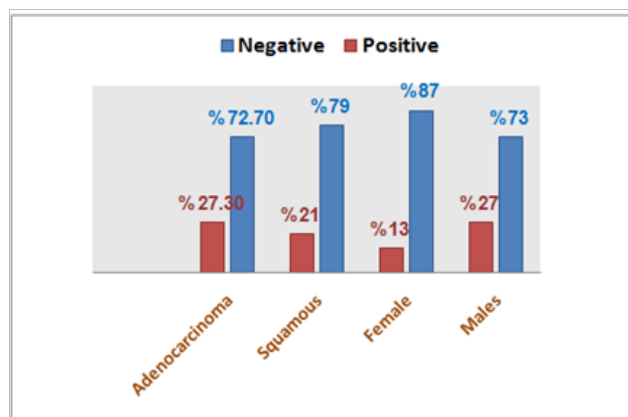
cancer, 15/55 (27%) were identified with EBV infection and the remaining 40/55(73%) found without EBV infection. Of the 46 females with esophageal cancer, 7/46(13%) were found positive for EBV immunostaining and the remaining 39/46(87 %) were found negative for EBV. The association of EBV risk with male sex was OR (CI)=2.08(0.77-5.7), P=0.145. According to cancer type, of the 91 cases of the Squamous cell carcinoma, 19/91(21%) were found with positive EBV and the remaining 72/91(79%) were negative. Of the 11 cases of the Adenocarcinoma, 3(27.3%) were found with positive EBV and the remaining 8(72.7%) were negative, as indicated in Table 1. The association of EBV risk with Squamous cell carcinoma was OR (CI)=0.70(0.17-2.9), P=0.63,as indicated in Table 1 & Figure 2.

**Table 1** Distribution of IHC results of EBV by sex and cancer type

Sex	Positive	Negative	Total
Males	15	40	55
Females	7	39	46
Total	22	79	101

Cancer Type	Positive	Negative	Total
Squamous	19	72	91
Adenocarcinoma	3	8	11
Total	22	80	102

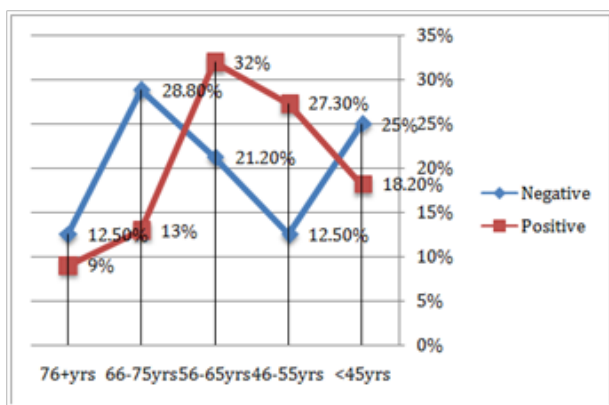


**Figure 2** Description of IHC results of EBV by sex and cancer type.

Concerning IHC staining results of EBV with age distribution, the highest positive results were found in age group 56-65years, representing 7/23(32%), followed by 46-55, <45, 66-75 and 76+ constituting 6/22(27.3%), 4/22(18.2%),3/22(13.5%) and 2/22(9%) respectively, whereas; the groups with the highest negative results were 66-75 years, representing 23/80(28.8%) followed by <45,56-65 and (46-55& 76+) constituting 20/80 (25%), 17/80(21.2%), and 10/80(12.5%) for each, respectively, as shown in Table 2 & Figure 3.

**Table 2** Distribution of IHC results of EBV by age

Age group	Positive	Negative	Total
<45 years	4	20	24
46-55	6	10	16
56-65	7	17	24
66-75	3	23	26
76+	2	10	12
Total	22	80	102



**Figure 3** Description of IHC results of EBV by age.

### Discussion

The prevalence rates of esophageal cancer vary according to geographic regions and related risk factors. Many risk factors, including EBV, may be involved in the development of esophageal cancer. Although there is no a clear-cut association between EBV infection and EC, but there is evidence that EBV infection may contribute to esophageal cancer in certain high-risk populations.<sup>12</sup> However, in the present study we found a prevalence of 21.5% of infection of EBV among Sudanese patients with EC. Relatively higher prevalence rates were previously reported in some studies. In study from German investigated 37 patients with esophageal carcinoma (Esophageal squamous cell carcinomas (n=23) and adenocarcinoma (n=14)) for the presence of human EBV DNA, EBV was detected in 35% of squamous cell carcinomas and 36% of adenocarcinoma.<sup>12</sup> Nevertheless in the present study EBV positive was found in 27.3% of the cases of the adenocarcinoma and 21% of the cases of SCC. In another study an association between EBV infection and the development of esophageal carcinoma has been reported in 35.5% of the cases.<sup>13</sup> Although, It is strongly suspected that the EBV plays a role in the genesis of nasopharyngeal carcinoma, but some studies reported that, EBV is infrequently associated with esophageal SCC, and may appear through tumor-infiltrating lymphocytes in some advanced lesions.<sup>14,15</sup> However, the Prevalence rates of EBV in gastro-esophageal cancer vary between 5 and 20%, which may depend on the patient groups in different studies.<sup>16-18</sup> EBV-associated tumorigenesis appears to be rather restricted to gastric cancer while the role

of EBV in other parts such as esophageal carcinomas appears to be insignificant in most parts of the globe.<sup>19</sup> To the best of our knowledge there no study investigated the role of the EBV in the etiology of EC from Sudan. Most of the studies in this context from Sudan, tested the association between EBV and nasopharyngeal carcinoma. Almost all of these studies found some degrees of association between EBV and nasopharyngeal carcinoma. In one study from Sudan, EBV genes were detected in 92/150 (61.3%) of patients with nasopharyngeal carcinoma.<sup>20</sup> Another study from Sudan has tested 43 Sudanese patients with nasopharyngeal carcinoma for the presence of EBV using EBER-ISH. All nasopharyngeal carcinoma biopsies (100%) were positive for EBER1 in almost all carcinoma cells.<sup>21</sup> These studies may give some degree of suspicion for EBV as possible etiological factor contributing to the etiology of EC in Sudan. In regard to the sex, the prevalence of EBV infection in the current study was 27% in males and 13% in females. Since there are only small studies in regard to the relationship between EBV and EC, the relationship of sex and EBV can't be strongly, but studies on the association of EBV and nasopharyngeal carcinoma indicates similar findings to our series, though the association between the number of males and females was not statistically significant. In regard to the age most of cases with positive EBV expression were found among age 56-65years. Although cancer in general accumulate at elderly people, but this need further research in regard to the start of infection and initiation of carcinogenesis.<sup>22,23</sup>

Esophagectomy is the leading treatment for esophageal cancer. Esophagectomy can be achieved by several diverse procedures, including McKeown (cervico-thoraco-abdominal), Ivor-Lewis (thoraco-abdominal), and transhiatal methodologies. It has been proposed that minimally invasive esophagectomy contributes to the decline of pulmonary complications. Cervico-thoraco-abdominal 3-field lymphadenectomy may extend survival, but a randomized control study on this matter has not been conducted. Death and disease rates after esophagectomy persist high. A number of meta-analyses verified that esophagectomy at low-volume hospitals was related with a significant increase in the incidence of in-hospital and 30-day mortality. The influence of hospital volume on long-standing outcome remains to be an issue of debate.<sup>24</sup> However, in the Sudan, there was great improvement in the early postoperative mortality from 27% in 1986 to 10% in recent reports. The surgical treatment presented a quick symptoms of relief which suited most patients coming from distant locations and couldn't afford to stay for extended periods in the Capital.<sup>12</sup> The limitations in the present study include; its retrospective settings and that fact it didn't measure other factors that contribute to the etiology of EC cancer, such as smoking, alcohol use, and other viruses (HPV). The lack of surgical management data was extra limitation in the present study. In conclusion: EBV might have some etiological effects contributing to the occurrence of esophageal cancer in Sudan. Crucial preventive strategies are extremely needed to reduce the burden of esophageal cancer in Sudan. Esophageal cancer is highly aggressive; consequently, a multimodality approach is essential to cure this disease. Surgical resection remains the backbone among these approaches.

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## Conflict of interest

The author declares no conflict of interest.

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