

## Association of EBV in Gastric carcinoma among Sudanese patients

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### Abstract:

**Background:** Gastric cancer is the second most common gastrointestinal cancer and is largely attributed to *Helicobacter pylori* (*H. pylori*) infection. In addition, studies have also shown association with Epstein-Barr virus (EBV) in 10% of gastric cancers. The aim of this review was to estimate the present of EBV in gastric cancer among Sudanese patients.

**Material and Methods:** In this is descriptive retrospective study, which carried out in Omdurman teaching hospital Khartoum-Sudan, involved 30 surgically treated formalin-fixed, paraffin embedded tumor samples from patients with gastric cancer, collected during January 2014 and October 2015. The presence of EBV in was determined using immunohistochemistry

**Result;** the study involved (30) subject 17 was male (56, 6) while 11 was female (36, 6) with (1.6: 1) males: female ratio, ten sample were positive (23.3) while 20 sample (76, 7) was negative, with no statistically significant difference between EBV infection and type of tumors.

**Conclusion:** high association of EBV and gastric cancer was reported in this study.

**Keywords:** Epstein – Barr virus; gastric cancer; LMP-1

### Introduction;

The Epstein-Barr virus (EBV), a member of the human herpes virus group, The EBV genome can be detected in malignancies of both lymphoid and epithelial cell origin(1). EBV genome in GCs was first detected in 1990 by Burke et al. using the polymerase chain reaction (PCR) technique(2), EBV-associated gastric carcinomas account about 10% of all gastric carcinomas worldwide(3). Gastric cancer is the third leading cause of cancer death in both sexes worldwide leading cause (723000 cases) of death annually (4). In Sudan incidence and prevalence are not clear because of absence of National Cancer Registry, First results from the Cancer Registry, 2009–2010 report gastric Cancer incidence (rate = 4.0 per 100,000)(5) Infection with *Helicobacter pylori* (*H. pylori*) is considered essential for the development of gastric cancer, such that *H. pylori* has been classified as a type I carcinogen by the International Agency for Research in Cancer (6) Other include, eating habits, particularly a diet rich in cured and smoked meat products, with low antioxidant content. Additionally tobacco smoking and alcohol consumption have a significant effect on the gastric cancer development.(7) The occurrence of EBV-associated

GC is estimated at more than 50,000 cases per year(8) is present predominately in men and in younger-aged individuals suggesting that risks related to lifestyle or occupational factors may exist among males, and presents a generally diffuse histological type(9).

### Material and Methods:

In this is descriptive retrospective study, which carried out in Omdurman teaching hospital Khartoum-Sudan, involved 30 surgically treated formalin-fixed, paraffin embedded tumor samples from patients with gastric cancer, collected during January 2014 and October 2015. Patient identification data were retrieved from patients records data include age, sex and histological type of the tumor

**Immunohistochemistry:** The immunohistochemical procedure was done as follows:

One section (3µm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto salinized slides (Thermo). Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and was placed in distilled water. Samples were steamed for antigen retrieval

for EBV using high PH (9) by water bath at 95°C. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and methanol for 10 min, and then Slides was incubated with (100 µl) of (mouse monoclonal antibody (CS1-4; Dako,) against LMP-1) (for 20 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 minutes with dextran labeled polymer ((Thermo -ultra vision). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides was counterstained with haematoxylin.. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

**Result:** The mean age of patients in this series was 54.5 years (range 15 – 80 years). The male to female ratio was 1.6: 1. Ten sample were positive (23.3) while 20 samples (76, 7) was negative. The EPV showed positive staining reaction in (3) cases of gastrointestinal stromal tumor, (3) cases of adenocarcinoma and 1 case of non-Hodgkin lymphoma, there was no relation between histological types of the gastric cancer and EPV expression, The P value was 0.459 which is statistically insignificant.

Table 1: The frequency of different histological types of gastric cancer:

Diagnosis	Frequency	Present
Adenocarcinoma	9	30%
gastrointestinal stromal tumor	14	46.7%
non-Hodgkin lymphoma	6	20%
Leiomyosarcoma	1	3.3%

Table 2: Relation between tumors type and EPV expression:

Type of Tumor	EBV expression		Total	P value
	Positive	Negative		
Adenocarcinoma	3	6	9	0.459
gastrointestinal stromal tumor	3	11	14	
non-Hodgkin lymphoma	1	5	6	
Leiomyosarcoma	0	1	1	
Total	7	23	30	

Table 4: Relation between EPV expression and the sex of patient

Gender	Expression		Total	P value
	Positive	Negative		
Male	4	13	17	0.653
Female	3	8	11	
Total	7	18	28	

**Discussion:** The relationship between Epstein-Barr virus and various epithelioid diseases has already been demonstrated. Involvement of EBV has been described in the etiopathogenesis of not only the nasopharyngeal carcinoma but other carcinomas as well, including gastric carcinomas.(10,11) A known contributing oncogenic factor is H. pylori infection. In fact, both EBV and H. pylori are considered class 1 oncogenic pathogens by the World Health Organization and are associated with overlapping subsets of gastric carcinoma (12, 13, and 14). It is estimated that EBV infection can be found in about 10% of GCs worldwide, especially those with marked lymphocytic infiltration (2.15). Many molecular changes such as DNA hypermethylation, role of EBV encoded mRNA and viral induced oncoproteins have been studied, but the exact pathogenesis is not yet fully understood (16, 17). LMP2A is reported to induce the phosphorylation of STAT3, which activates DNA methyl transferase 1 (DNMT1) transcription and causes loss of PTEN expression through CpG island methylation of the PTEN promoter in EBV-associated GC (14) LMP1 can also induce aberrant DNA methylation by activating DNMT1 through the JNK signaling pathway and inducing DNA methylation of host cells (18).

The current study found that there was male predominance with a ratio of almost 1.6:1; this preponderance is not unexpected considering that gastric cancer is more common in men. Apart from the obvious gender difference, other risk factors include lifestyle and occupational factors, intrinsic biological and hormonal factors (19).

Our study showed that the estimated point prevalence of EBVaGC was 23.3%, much higher than rates reported from other countries, generally around 10% of all gastric cancer (20,21,9) .others authors report 18% as in Germany(22) Galetsky et al. reported a frequency of 8.7%. In Russia, (23) In the US and Mexico, 8.15 to 16% of gastric carcinomas were EBV positive (3, 24).

**In conclusion,** it is clear that EBV is associated with gastric carcinomas and positive rate for EBV, higher than what have been reported in the literature

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