

Original Research Article

Frequency of Epstein Barr virus infection among Sudanese children patients with Burkitt's lymphoma

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ABSTRACT

Background: Burkitt's lymphoma (BL) is a B-cell malignancy categorized in three forms endemic, sporadic, and immunodeficiency-associated variants. Epstein barr virus (EBV) is associated with BL in almost all African populations. The frequency of EBV associated with BL is unknown in Sudan. The study aimed to estimate the frequency of EBV in Sudanese patients with BL.

Methods: The study was a descriptive cross-sectional study conducted between June 2014 and July 2019 in 34 paraffin-embedded tissue sections obtained from diagnosed BL patients attending Soba university hospital in Khartoum city (Sudan). Monoclonal IgG1 antibody was used to detect EPV by immunohistochemistry technique.

Results: The study showed that 44.1% (15/34) of BL patients were positive for EBV, whereas 55.9% (19/34) showed negative EBV. Males, 66.7% (10/15) were more affected by the EBV infection than females, 33.3% (5/15). P value=0.014.

Conclusions: Despite BL is considered a rare type of lymphoma in Sudan; the obtained results showed a lower frequency of EBV compared with other studies worldwide.

Keywords: Frequency, Epstein barr virus, Burkitt's lymphoma, Immunohistochemistry test

INTRODUCTION

Burkitt's lymphoma (BL) is a type of B- cell tumor with aggressive progression, which occurs most frequently in children.¹ It is characterized by high proliferative of B cells, which appear in a basophilic cytoplasm and medium size.² The incidence of BL is high among African children, about 3–6 new cases per 100,000. This high incidence is related to areas where *Plasmodium falciparum* malaria is widespread.³ BL is caused by a translocation of the c-MYC gene at 8q24 with the heavy chain immunoglobulin (IGH) gene on 14q32 (80%) or, less frequently, with the κ light chain locus (IGK) at 2p11

(15%) or the λ light chain locus (IGL) at 22q11 (5%).⁴ Based on epidemiological observations, BL is classified into three groups; endemic BL (eBL), sporadic BL (sBL), and HIV-associated BL.¹ The eBL type is associated with (EBV) in more than 95 % of cases and is predominant in all parts of the world where malaria is hyperendemic.⁵

Epstein Barr virus (EBV) is a double-stranded DNA virus that belongs to viridae family. It is characterized by a tropism for B-lymphocytes displaying latent infection in the host.⁶ EBV is found in many malignancies; the virus can turn the B lymphocytes into immortalized cells such as in BL.⁷ This makes the International agency for

research on cancer (World health organization [WHO], 1997; WHO, 2011) classified EBV as a class I carcinogen.⁸

The frequencies of EBV in BL differ depending on the clinical variables of the disease. In the endemic type of BL, EBV is present in most cases, while in sporadic BL, the frequency is fewer than 30% of cases and between 30-40% in AIDS-related BL.⁹

EBV-positive Burkitt's lymphoma has a higher frequency than EBV-negative disease of somatic mutations in the immunoglobulin variable heavy chain with evidence of antigen selection.¹⁰

In Sudan, the frequency of EBV among patients with BL has not been systematically evaluated and underestimated. Sudan is known as one of the endemic areas of malaria in Africa. Measuring the EBV frequency is an essential step in using the appropriate treatment and determining effective prevention, control, and care methods. This study was designed to assess the frequency of EBV among Sudanese children with BL.

METHODS

This is a descriptive cross-sectional study conducted between June 2014 and July 2019 in 34 newly diagnosed patients (61.8% were Males and 38.2% were females) with a Burkitt's lymphoma attending Soba university hospital in Khartoum city (Sudan). Their age ranged (2-13 years old); any diagnosed patients with Burkitt's lymphoma confirmed by immunohistochemistry have been included in the study, while any patient under treatment and not confirmed by immunohistochemistry for Burkitt disease have been excluded. Samples were obtained in Paraffin-embedded tissue blocks. Control samples from healthy individuals were run with patients' samples.

Tissue microarray (TMA) was done according to the conventional protocol.¹¹ In brief, the recipient block with 34 square lattices (2 mm in width) was first prepared. A tissue rod was then crafted from each paraffin-embedded patient block and arrayed manually into a pre-softened recipient block. TMA block was placed onto a glass slide to facilitate the bonding of the patient's cores with the recipient block's paraffin. After that, a total of 34 TMA sections (4 µm thick sections) could be easily obtained from a TMA block using the microtome. The section is placed on a super frost glass slide and can be used in immunohistochemistry (IHC) to detect EBV.

Immunohistochemistry detection of EBV latent membrane protein

EBV was detected using the monoclonal IgG1 antibody for EPV-latent membrane protein (LMP) (clone: CS1/CS2/CS3/CS4) reagent kit manufactured by ZYTOMED systems GmbH. This antibody is a mixture

of 4 antibodies that detect the different epitopes of Epstein Barr virus latent membrane protein (LMP). In brief, peroxide blocking (3% H₂O₂ solution) was applied onto paraffin-embedded tissue sections for 10 min before washed with a buffer for 2 min. The section was covered with a blocking solution for 5 min after washing with a 1% PBS buffer for 2 min. Then a primary antibody (optimally diluted) was added for 45 min, then washed with buffer three times for 5 min. after that HRP one-step polymer anti-mouse was applied for 30 min, and washed with buffer three times for 2 min. Then DAB was added for 5-15 min. The reaction is then stopped with distilled H₂O when the desired color intensity is attained.

Lastly, counterstaining and bluing was performed, and the section was mounted: aqueous with AEC and permanent with DAB. EBV positive lymphoblastoid cell lines were stained by brown color (Figure 1). Frequency distribution and Chi-square obtained by SPSS version 21. The confidence interval (CI) was set at 95%.

The institutional review board has approved the study for Ethical clearance. Furthermore, written informed consent was obtained from all the children's parents before sample collection.

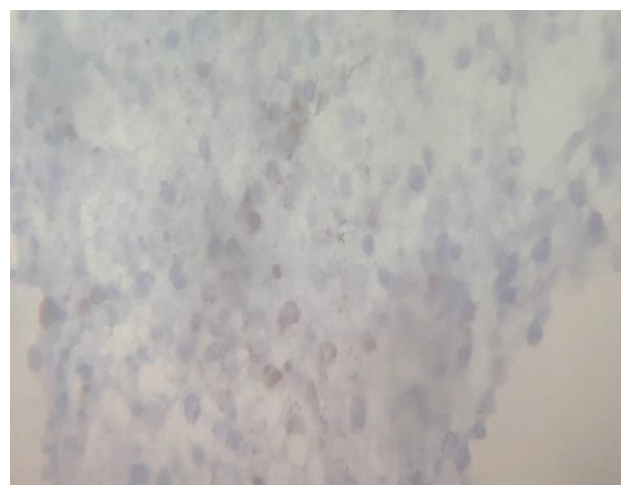


Figure 1: Positive EBV immunohistochemistry from BL patient.

RESULTS

A total of 34 Sudanese children (61.8% males and 38.2% females) with Burkitt's lymphoma were enrolled in this study.

Based on the immunohistochemistry detection of Epstein-Barr Virus, the study showed that 44.1% (15/34) of BL patients were positive for EBV, whereas 55.9% (19/34) showed negative EBV. Also, chi-square showed that males 66.7% (10/15) were more affected by the EBV infection than females 33.3% (5/15), p value=0.014 (Table 1).

Table 1: Frequencies of EBV among different gender.

EBV	Male N (%)	Female N (%)	Total	P value
Positive	10 (66.7)	5 (33.3)	15 (44.1)	0.014
Negative	11 (57.9)	8 (42.1)	19 (55.9)	
Total	21 (61.8)	13 (38.2)	34	

DISCUSSION

Burkitt Lymphoma (BL) is a type of B-cell malignancy. It occurs most predominantly in children, especially in malaria areas with less prevalence in other parts of the world.¹² EBV infection had a role in the pathogenesis of Burkitt's lymphoma.¹³ As the frequency of EBV in BL is unknown in Sudan, this study was aimed to estimate it. Our study revealed agreement in the frequency EBV's in Sudanese children with Giuseppe P in Italy (47.4%) and Parviz in Iran (50%).^{14,15}

At the same time, it disagreed with studies done in Brazil by Araujo, Sandlund et al and Eduardo, in which they reported the frequency of EBV in BL as 87%, 73%, and 63%, respectively.¹⁶ As EBV is less frequent in sporadic BL, this might reduce the frequency in our study.

Yousif et al studied the frequency of EBV in 90 Sudanese patients (70 were malignant lymphoma, and 20 were lymph node hyperplasia). He noticed positive EBV in 32% of malignant lymphoma and 10% of lymph node hyperplasia; this indicated that EBV might inter in the pathogenicity of lymphoma in Sudan.¹⁷

Moreover, the study showed that positive EBV in BL was more significant in males than females; the ratio was: 3:1, this finding was agreed with a study done by eduardo. The reason for this gender domination in BL may be due to genetic and hormonal factors.¹⁶

CONCLUSION

Despite BL is considered as a rare type of lymphoma in Sudan; the obtained results showed a lower frequency of EBV compared with other studies worldwide. The research will also illustrate the importance of EBV screening among BL patients to use the best medication to avoid the appearance of a complication of the disease.

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