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# Frequency Rate of Epstein-Barr Virus Viraemia among Sudanese Patients presenting with Acute Febrile Illnesses in Khartoum State

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## Abstract

**Background:** Acute febrile illnesses (AFIs) are defined as non-specific illnesses presenting with fever ≥38°C and lasting for less than two weeks without a readily diagnosable source after routine clinical evaluation. AFIs are the most common causes of outpatient attendance and mortality, especially among children. Studies that have attempted to identify pathogens associated with AFIs and several etiologies had been reported.

**Objective:** To estimate the frequency rate of Epstein-Barr virus (EBV) viraemia among Sudanese patients presenting with acute febrile illnesses in Khartoum State.

**Materials and methods**: Under aseptic condition, 4 ml of venous blood were collected in sterile EDTA containers from 50 patients presenting with acute febrile illnesses other than malaria. EBV was detected in these blood specimens using qualitative molecular method targeting a highly conserved BamH1W gene.

**Results:** The overall frequency rate of EBV viremia estimated in the study population was 8%, among adults more than 15 years, 4% among children aged less than 5 years, and 2% among adolescents aged 5-15 years. This difference was insignificant ($p = 0.950$). With respect to the geographical localities, the frequency rate of EBV viremia was 6% in Khartoum, and 2% in Omdurman. Patients from malaria endemic regions presented with higher viremia than those from other regions. EBV genome detected was higher in females (6%) than in males (2%).

**Conclusion:** The risk of EBV viraemia increases with older age.

**Keywords**: Epstein-Barr virus viraemia, Acute febrile illnesses, BamH1W gene.

## Introduction

Epstein-Barr virus is a member of the human herpes viruses. Most acute febrile illnesses (AFI) are not associated with a specific diagnosis due to limitation of proper diagnostic tools. In malaria endemic regions, AFI, especially in children, are clinically treated as malaria cases even if the parasite was not detected. This leads to over-diagnosis of malaria cases. A better understanding of the causes of non-malaria fever is required to guide effective clinical management. EBV may account for a significant occurrence of AFI cases. Symptomatic EBV infection is commonly characterized by febrile episodes, and has many clinical signs that cannot be differentiated from those of other febrile illnesses[1].

EBV is one of the most common human viruses, and it is found all over the world. Recent studies have shown that EBV seroprevalence rate is estimated to be more than 90% among adults older than 35 years of age worldwide. Each year, new infections are estimated as 200,000 cases. The Epstein-Barr virus is commonly acquired during childhood in developing countries, and among more than 90% of pre-school children. In developed countries, many people are not infected in childhood but are rather infected in adolescence or during adult-hood. Variations in the EBV genome made it possible to distinguish two subtypes of the virus: EBV-1 and EBV-2 (or EBV-A and EBV-B types)[2].

Seroprevalence studies have shown that EBV-1 strain predominates in western countries, whereas EBV-2 strain is only common in some areas of equatorial Africa and New Guinea. Primary infection with EBV is often asymptomatic or is responsible for infectious mononucleosis and generally with no serious complications. On the other hand, chronic infection is reported in many cases of gastric carcinoma, Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's classic lymphoma, and oral hairy leukoplakia. Furthermore, it is suggested that EBV is associated with brain cancer, salivary gland tumors, hepatocellular carcinoma, and multiple sclerosis especially in immunocompromised individuals[3].

## Materials and methods

This was a descriptive cross-sectional study conducted in out-patient clinics and health centres of two hospitals in Khartoum State (Sudan), to detect Epstein-Barr virus in patients with acute febrile illnesses. The study was approved by the Scientific Research Committee of Al Neelain University. Data confidentially was maintained, and the specimens and information collected from all participants had not been used for any purpose other than this study. Permission to collect the specimens was granted from hospital authorities in Khartoum State. All study population had been informed with this study and a formal consent was obtained from each one of them. Clinical and demographical data (age and gender) was collected by a structured questionnaire.

The study population were adults above 15 years, children under 5 years, and children 5-15 years. All patients investigated were presenting with acute febrile illnesses (temp. ≥ 38°C). Patients were screened for malaria and whose found without malaria were enrolled in the study. 3 ml of whole blood were collected under aseptic technique from all participants in sterile EDTA containers using the salting out method. 300 µl of sample was added to Eppendorff tubes and used for DNA extraction by the polymerase chain reaction (PCR) technique.

DNA extraction: DNA was extracted by adding 1ml red blood cell lysis buffer to the EDTA whole blood, vortexed for 20 seconds and centrifuged at 2500 rpm for 10 minute. Buffer was discarded and the remaining column was span and centrifuged at 2500 rpm until a white pellet was obtained. Then 300 µl white blood cell lysis buffer and10 µl proteinase K were added and incubated at 37°C for one hour. Then 100 µl saturated NaCl (6M) were added to each tube and shaked for 15 seconds, 200 µl cold chloroform were added, and the mixture was centrifuged at 1800 rpm for 6 minutes. After that the aqueous phase was transferred to a clean Eppendorff tube by pipetting. The tube was then centrifuged at 1400 rpm for 5 min. and the supernatant was

discarded without disrupting the precipitate, washed with 600 µl 70% ethanol, and centrifuged at 6000 rpm for 5 min. to elude the DNA pellet which was resuspended in 50 µl TE buffer.
PCR technique: This was performed by processing the extracted DNA with the primers that were obtained from Macro.Gen.Bioteth (Korea) as follows:
* Forward primer: 5'-GCA GCC GCC CAG TCT CCT-3'
* Reverse primer: 5'-ACA GAC AGT GCA CAG CCT-3'
The master mix was 5x FIREPOl®.
The template DNA, primer, and water were added to 5x FIREPOl®. Then 2 µl of primer (sense and antisense) and distilled water were added into the tubes to give a total volume of 20ml. PCR was started with initial denaturation 94$^{o}$C for 10 minutes. PCR cycles were conducted by denaturation at 94$^{o}$C for 45 seconds, annealing at 60$^{o}$C for 1min., and extension at 72$^{o}$C for 45 seconds. Final cycle extension was performed at 72$^{o}$C for 10 min. The PCR product was run on 1.5 agarose gel electrophoresis containing ethidium bromide and the loading dye was added to the ladder, DNA was visualized under UV light using the gel documentation system.
Data was analyzed using the statistical package of social science (SPSS) program version 25. The results were expressed as frequencies and percentages. The descriptive statistics was obtained to calculate the frequency and percentage of study variables. Chi-square test was done to study the association between *BamH1W* gene and the study variables (age, gender, residence) Significance was considered at p-value = $\leq 0.05$.

## Results

In the present study we aimed to detect EBV DNA in whole blood samples by the standard non-quantitative conventional PCR. This technique is able to detect 50 viral copies of a specific fragment of the expected size by using the primer set specific to the EBV.
A total of 50 patients presenting with non-malaria, acute febrile illnesses were investigated for presence of EBV DNA.
Furthermore, regarding EBV genome prevalence rate among acute febrile illnesses patients, 4 patients (8%) were positive and 46 patients (92%) were negative.
As shown in Table (1), EBV genome was detected among 29 patients (58%) resident in Khartoum; and among 21 patients (42%) resident in Omdurman. This difference was statistically insignificant (p = 0.436). Also, EBV DNA was detected among 4 patients (8%) aged more than 15 years old, among 14 patients (28%) aged 5-5 years old, and among 10 patients (20%) aged less than 5 years old. This difference was statistically insignificant (p = 0.823 and 0.950 respectively). On the other hand, EBV genome was positive among 24 male patients (48%) and 26 (52%) female patients. This difference was statistically insignificant (p = 0.336).

## Discussion

EBV is a common viral infection, and its symptoms often resemble malaria and other febrile illnesses. The goal of this study was to determine the EBV virus in patients with febrile illnesses that could not be attributed to malaria. In this study, EBV DNA in 4 patients (8.0%). Use of PCR facilitated the detection of the virus. There was no significant difference in the prevalence rate of positive EBV between males and females (p = 0.336).

The prevalence rate of positive EBV reported in this study was (8.0%). It was in disagreement with that obtained by other workers[4] who reported a higher rate (29.2%). This may be due to the large sample size they analyzed in their study.
Most studies of EBV has focused on the association of EBV with malaria in the causation of Burkitt's lymphoma. Two recent seroprevalence studies were performed in USA by the National Health and Nutrition Examination Surveys (NHANES), in individuals ranging from 6-19 years

Table (1): Distribution of EBV genome according to residence, gender, and age incidence

| Parameter | Positive EBV | Negative EBV | Total | P - value |
|---|---|---|---|---|
| Khartoum residence | 3 (6%) | 26 (52%) | 29 (58%) | 0.436 |
| Omdurman residence | 1 (2%) | 20 (40%) | 21 (42%) | |
| Female | 3 (6%) | 23 (46%) | 26 (52%) | 0.336 |
| Male | 1 (2%) | 23 (46%) | 24 (48%) | |
| Under 5 years | 1 (2%) | 9 (18%) | 10 (20%) | |
| 5-15 years | 1 (2%) | 13 (226%) | 14 (28%) | 0.823 |
| Above 15 years | 2 (4%) | 24 (48%) | 26 (52%) | 0.950 |

showed that EBV seroprevalence ranging from 50% in 6-8 years, 55% in 9-11 years; 59% in 11-14 years; 69% in 15-17 years; and 89% in 18-19 years[5].
In terms of geographical disparity, frequency rate of EBV viremia was higher in Khartoum (6.0%) than in Omdurman (2.0%). Many factors could be responsible for the geographical trends, including human social behavior as well as economic activity[5 - (35)].
This factors may determine person-to-person contact and may play a role in viral transmission. Crowding specially in social events is a known risk factor for EBV infections. There was no variation on the infection with EBV in females compared to that of males (6.0% vs. 2.0%; $p$ = 0.336) (odd ratio = 3.000)[6].
Other reports have showed a decrease in seroprevalence rate of EBV with age. This discrepancy is due to the method used, in endemic areas it was not possible to rule out increased viremia from reactivation by other diseases. Our data on EBV viremia is inconclusive on the clinical importance of finding EBV in the blood we think this is because our tests for pathogen were not exhaustive and we do not rule out other cause of upper tract infections .further in depth studies including large sample size and other area are recommended[7].
Conclusion: The risk of EBV viraemia increases with older age.

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