International Journal of Scientific Research in Science, Engineering and Technology
Print ISSN: 2395-1990 | Online ISSN : 2394-4099 (www.ijsrset.com)
doi : https://doi.org/10.32628/IJSRSET229650

Molecular Detection of Epstein Barr Virus in Malaria Positive Patients in Khartoum State Hospitals, Sudan 2018

Khalid A. Abbas[1], Reham A. Alaidros[1], Salah aldeen Dahawi[1], Altayib Z.Mohammed[1], Awadalkareem Y. Awadalkareem[1,2] Mutaz A Elsir[1,2], Abdel Rahim M El Hussein[2], Isam M. Elkhidir[2] , Khalid A. Enan[2*]

[1]AL Neelain University, Facutly of Medical Laboratory Sciences, Medical Microbiology Department, Khartoum, Sudan.

[2] Central Laboratory, Ministry of High Education and Scientific Research, Khartoum, Sudan

*Corresponding Author : Khalid A Enan, Central Laboratory, Ministry of High Education and Scientific Research, P.O.Box:7099, Khartoum, Sudan

Email : khalid.enan@gmail.com.

Article Info
Volume 9, Issue 6
Page Number : 317-322

Publication Issue :
November-December-2022

Article History
Accepted : 01 Dec 2022
Published: 15 Dec 2022

ABSTRACT

Background: Malaria and Epstein–Barr virus (EBV) infections are recognized cofactors in the genesis of endemic Burkitt lymphoma, the most common pediatric cancer in equatorial Africa.

Objective: To detect Epstein - Barr virus in malaria positive patients in Khartoum state

Materials and methods: A cross-sectional study was conducted in Khartoum State hospitals during the period from April 2018 to April 2019. Fifty specimens from malaria positive patients (16 males and 34 females) were collected and tested by conventional polymerase chain reaction (PCR) for detection of EBV.

Results: Out of the 50 malaria positive patients samples tested by PCR, EBV was detected in 5 (10%) samples.

Conclusion: The rate of EBV infection among Sudanese malaria patients was low (10 %, 5/50).

Keywords: malaria parasite, polymerase chain reaction, Epstein-barr virus

I. INTRODUCTION

Epstein–Barr virus (EBV or human herpes 4) is a human herpes virus that establishes a persistent infection in 90% of the world's population. Similar to other herpes viruses, EBV has two modes of lifestyle: latent or (non-productive) infection, and lytic or (productive) replication. Following primary early life infection, EBV persists within memory B lymphocytes in a dormant state for the lifespan of the host. A low level of reactivation into lytic replication allows viral excretion into the saliva leading to contact transmission of the virus in vivo [1].

The lifelong permanent infection established by EBV is harmless in almost every host and rarely causes disease, unless the host–virus stability is upset. So, viral persistence represents a balance between viral dormancy, viral active replication, and host immune

responses. The lytic phase of viral replication can be caused in vitro by a variety of reagents and stimuli, including halogenated pyrimidine [2], phorbol esters [3], calcium ionophores [4], transforming growth factor b [5], butyrate [6], and triggering of the B cell receptor (BCR) with anti-immunoglobulin (anti- Ig) antibody (Ab) [7]. Less is known about the physiological stimuli that control activation of the virus productive cycle in vivo, though replication seems to take place following plasma cell differentiation [8].

It has been well documented that EBV is causally associated with various malignancies, including endemic Burkitt lymphoma (BL), nasopharyngeal carcinoma, and B cell lymphoma, in immunocompromised hosts [9].

Both EBV infection and intense exposure to Plasmodium falciparum malaria; in holoendemic malaria; are known cofactors in the pathogenesis of BL, which is the commonest pediatric cancer in equatorial Africa, accounting for up to 74% of childhood malignant disorders [10].

Development of BL, a B cell malignancy, is signaled by high Ab titers to antigens indicative of EBV reactivation [11].

Recent reports indicate that the impact of malaria infection on EBV persistence is reflected by an increased viral replication. Children living in endemic malaria areas have higher EBV load [12,13], and acute malaria infection leads to increased levels of circulating EBV that are cleared following anti-malaria treatment [14].

The mechanisms that may lead to viral reactivation during P. falciparum malaria are poorly understood. The identification of a polyclonal B cell activator and Ig binding protein in *P. falciparum* is of particular relevance. It was shown that the cystein-rich inter-domain region 1a (CIDR1a) of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) induces proliferation and activation of B cells, particularly of the memory subset, where EBV resides [15, 16].

In the 1970s, IARC demonstrated that more than 90% of adults worldwide are infected with EBV, based on the detection of antibodies to EBV (especially antibodies to viral capsid (VCA) and complement-fixing soluble (CF/S) antigens) [17] Many other epidemiological studies have shown since that EBV is highly prevalent throughout the world [18,19] ,including in isolated populations [20, 21].

Sudan is highly endemic malaria country; over 1.8 million cases of malaria were reported from across Sudan in 2019[22].

On the other hand, few scattered studies have been conducted in several Sudanese subpopulations using different types of samples and methods to detect EBV infection. For example,

Jalouli et al ` (2010) tested oral brush samples from apparently healthy volunteers and in patients with oral cancer ,EBV was detected in 118/175 (67.4%) of healthy people and in 69/217(31.8%) of the oral cancer patients samples[23].

In a more recent study 6 (5.88%) out of 102 esophageal cancer patients were found to be positive for EBV by PCR [24].

Only 3 (7%) out of 42 ductal breast carcinoma were positive for EBV DNA and none of 18 benign breast tumor (control group) were positive for EBV DNA [25].

In yet another study the results showed that eight out of 70(11.4%) breast cancer (Osman et al, 2019) patients were positive for EBV virus [26]. In contrast, a high frequency of 55.5% of EBV DNA detection in women with breast cancer has been reported.

In a relatively larger study among nasopharyngeal carcinoma patients in Sudan EBV genes were detected in 92/150 (61.3%) tissue samples using PCR [27].

On the other all 43 samples of NPC (100%) were found positive for EBV by in situ hybridization [28]. Further, EBV LMP1 gene transcripts were found in 29 (36.3%) of the 80 patients with paedriatic leukemia but in none of the healthy controls ($P < .0001$) [29].

To the best of our knowledge no studies have addressed EBV infection in malaria afflicted patients in Sudan.

II. METHODS AND MATERIAL

Study Design

This is a cross sectional study carried out in Khartoum state's hospitals that was conducted during period April2018 to April 2019.

Clinical samples

All participating patients were given a written informed consent. Blood samples from 50 patients with microscopically confirmed malaria parasite (16 males and 34 females) were collected in EDTA tubes. The blood samples were centrifuged at 3000 RPM for 5 minutes and obtained plasma was then used for DNA extraction.

DNA extraction

DNA extraction was done by Saturated sodium chloride method as mentioned by Miller et al [30]. 300 µl of blood samples were resuspended in 1.5 ml Eppendorf's tube with 1000 µl red cell lysis buffer (RCLB), mixed well and centrifuged at 5000 rpm for 10 min, Supernatant was discarded and 300 µl of white cell lysis buffer (WCLB) was added, 10 µl of 10% SDS and 20 µl of protein's K solution were then added and the mixture was incubated for 1 h at 65°C. Then 100 µl of 6M NaCl was added followed by 200 µl of cold chloroform and centrifuged at 18000 rpm for 6 min supernatant containing the DNA was then transferred to a new tube the viral DNA was finally eluted in 25ul of elution buffer and stored at -20^{0}C

Polymerase Chain Reaction (PCR):

The PCR was performed using primers that are specific for the EBV primers for EBV sequence were (SL1, 5-GGACCTCAAAGAAGAGGGGG-3 and the reverse primer SL3, 5-GCTCCTGGTCTTCCGCCTCC-3). The reaction was performed in 20 ul total volume that included 5ul master mix, 1ul forward primer, 1 ul reverse primer, 4 ul extracted DNA and 9ul distilled water.

The cycling condition were as follow:

Initial denaturation at 94 for 10min followed by 40 cycles of denaturation at 94°C for 1min annealing at 60 °C for 45s and extension at 72°C for 45s with final extension at 72 for 1 min ,10 ul of amplified product was analyzed by gel electrophoresis in 2% agarose stained with 0.15% ethidium bromide and visualized by using UV gel documentation system The expected size for EBV GENE amplicon was 80 bp.

Statistical analysis

Cramer test and SPSS 16 software were used to perfor m the analysis.

Statistical significance was set at a P value of less than 0.05.

Ethical Review

The study was approved by the Ethical Review Committee (ERC) of Alneelain University, the Ministry of Higher Education & Scientific Research, Khartoum State, Sudan.

III. RESULTS AND DISCUSSION

Results

Out of 50 malarias positive patient tested, 5 (10 %) were found positive for EBV DNA by PCR (table. 1). Based on age group, the distribution of patients positive for EBV were (60%) and (40%) in the age groups 20-40 year and 41-60yraes respectively (p value 0.083) (table.2)

According to gender, EBV was positive in (18.7%, 3/16) of the male patients and (5.8%, 2/34) of female patients but with no significant difference between male and female (P value 0.512). (table.3)

Table 1: prevalence of EBV in studied groups

Malaria positive patients	Positive PCR for DNA of EBV		Negative PCR for DNA of EBV	
Total NO.	No.	%	No.	%
50	5	10	45	40

Table 2: prevalence of EBV according to age groups

Age	0-20	21-40	41-60	61-80	P value
Positive	0	3	2	0	0.083
Negative	7	18	15	5	

Table 3: prevalence of EBV according to gender

Gender	Male	Female	P value
Positive	3 (18.7%)	2(5.8%)	0.512
Negative	13(81.3%)	32(94.2%)	

Discussion

EBV is a potent transforming virus for human B cells that has been implicated in the pathogenesis of numerous malignancies. Worldwide, over 90% of people are infected with EBV during their lifetime[31]. . After infection, EBV persists in a latent state throughout life, selectively in memory B lymphocytes. Immunosupression as a result of diseases such as HIV or malaria can lead to the reactivation and proliferation of EBV-infected cells [32]. EBV infected people have a higher prevalence of *P. falciparum* malaria and a higher *P. falciparum* genetic diversity score than controls[33],but the relative role of the malaria parasite and EBV in the development of BL remain unclear.

Number of studies has reported a positive correlation between EBV and malaria positive patients, in N Rasti et al 2005 EBV DNA is detected in 47% in the malaria infected [34]

The frequency of EBV detection in malaria positive patients is varied in different regions of world. In the present study only five (10%) of our malaria positive sample were positive for EBV DNA.

These results indicated that malaria may not play a significant role in concurrent EBV reactivation in patients from Khartoum. This is in contrast with Moormann, et al (2005,2011) statements that *P. falciparum* malaria induces polyclonal B-cell expansion and EBV reactivation that can increase the likelihood of a *c myc* oncogene translocation, and hence the development of BL; or that malaria infection weakens EBV-specific T-cell immunity, leading to reactivation of EBV in infected B cells and the loss of viral control[12,35]. \ It is also in contrast with the suggestion that malaria has inherent effects on EBV–host balance that contribute to great increase in viral load present in children from malaria endemic areas and probably result in an increased risk for BL[36].

IV.CONCLUSION

In the present study only 10% of malaria positive patients showed EBV infection using PCR, which is low compared to EBV infection rates in other subpopulations tested in Sudan. No significant differences according to age and sex were discernible. Finally, our study represents the first report on EBV infection in malaria patients in Sudan.

V. REFERENCES

[1]. Rickinson AB, Kieff E (2001) Epstein-Barr virus. In: Field's virology. 4th edition. Philadelphia: Lippincott-Raven. pp. 2575–2627.

[2]. Derge JG, Martos LM, Tagamets MA, Chang SY, Chakrabarty M (1973) Identification of a critical period during the S phase for activation of the Epstein-Barr virus by 5-iododeoxyuridine. Nat New Biol 244: 214–217.

[3]. zurHausen H, O'Neill FJ, Freese UK, Hecker E (1978) Persisting oncogenic herpesvirus induced by the tumour promotor TPA. Nature 272: 373–375.

[4]. Faggioni A, Zompetta C, Grimaldi S, Barile G, Frati L, et al. (1986) Calcium modulation activates Epstein-Barr virus genome in latently infected cells. Science 232: 1554–1556.

[5]. di Renzo L, Altiok A, Klein G, Klein E (1994) Endogenous TGF-beta contributes to the induction of the EBV lytic cycle in two Burkitt lymphoma cell lines. Int J Cancer 57: 914–919.

[6]. Luka J, Kallin B, Klein G (1979) Induction of the Epstein-Barr virus (EBV) cycle in latently infected cells by n-butyrate. Virology 94: 228–231.

[7]. Takada K (1984) Cross-linking of cell surface immunoglobulins induces Epstein-Barr virus in Burkitt lymphoma lines. Int J Cancer 33: 27–32.

[8]. Laichalk LL, Thorley-Lawson DA (2005) Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. J Virol 79: 1296–1307.

[9]. Thompson MP, Kurzrock R (2004) Epstein-Barr virus and cancer. Clin Cancer Res 10: 803–821.

[10]. Magrath I (1990) The pathogenesis of Burkitt's lymphoma. Adv Cancer Res 55: 133–270.

[11]. Henle G, Henle W, Clifford P, Diehl V, Kafuko GW, et al. (1969) Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. J Natl Cancer Inst 43: 1147–1157.

[12]. Moormann AM, Chelimo K, Sumba OP, Lutzke ML, Ploutz-Snyder R, et al. (2005) Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. J Infect Dis 191: 1233–1238. 13. Rasti N, Falk KI, Donati D, Gyan BA, Goka BQ, et al. (2005) Circulating Epstein-Barr virus in children living in malaria-endemic areas. Scand J Immunol 61: 461–465.

[13]. Donati D, Espmark E, Kironde F, Mbidde EK, Kamya M, et al. (2006) Clearance of circulating Epstein Barr virus DNA in children with acute malaria after antimalaria treatment. J Infect Dis 193: 971–977.

[14]. Donati D, Zhang LP, Chen Q, Chene A, Flick K, et al. (2004) Identification of a polyclonal B-cell activator in Plasmodium falciparum. Infect Immun 72: 5412–5418.

[15]. Donati D, Mok B, Chene A, Xu H, Thangarajh M, et al. (2006) Increased B cell survival and preferential activation of the memory compartment by a malaria polyclonal B cell activator. J Immunol 177: 3035–3044.

[16]. de The G, Day NE, Geser A et al. (1975).Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study - a review. IARC SciPubl, 11: 3–16.

[17]. IARC. (1997) Epstein-barr virus and Kaposi's sarcoma herpesvirus/Human herpesvirus 8. IARC MonogrEvalCarcinog Risks Hum, 70: 1–492. PMID:9705682

[18]. Young LS. (2008) Epstein-Barr Virus: General Features. In: Encyclopedia of Virology. Mahy BWJ, van Regenmortel MHV, editors-in-chief. Oxford: Academic Press, pp. 148–157.

[19]. Black FL, Woodall JP, Evans AS et al. (1970). Prevalence of antibody against viruses in the Tiriyo, an isolated Amazon tribe. Am J Epidemiol, 91: 430–438. PMID:4316199

[20]. Tischendorf P, Shramek GJ, Balagtas RC et al. (1970) Development and persistence of immunity to Epstein-Barr virus in man. J Infect Dis, 122: 401–409. PMID:4320006

[21]. Anon, (2019) OCHA, Sudan, Situation Report .Last updated: 12 Dec 2019 Downloaded 1-5-2022

[22]. Jamshid Jalouli, Salah O. Ibrahim, Dipak Sapkota, Miranda M.Jalouli, Endre N. Vasstrand, Jan M.Hirsch, Per-Anders Larsson, (2010) presence of HPV, HSV,HBV DNA in oral biopsies from Sudanese patients

[23]. HG Ahmed,MABI Beely, MSAE Aziz, BA ALmutlaq, MZ Alturkstani. (2017) screening of Epstein-barr virus in esophageal cancer patients in Khartoum state sudan.MOJ Surg 4(6),00092

[24]. Riham E A, Abdel Rahim M El H, Salahaldeen H D, Isam M Elkhidir, Khalid A Enan. (2018) Molecular Detection of Epstein-Barr Virus and Human Cytomegalovirus Antigen Expression in Breast Cancer in Khartoum State, Sudan. Nov Appro in Can Study.3(2).NACS.000560.2019. DOI: 10.31031/NACS.2019.03.000560

[25]. Osman IM, El Hussein AM, Elkhidir IM, BabikerA, Enan KA. (2019) Molecular Detection of Epstein Barr Virus,Human Papilloma Virus

Types 16,18 in Breast Cancer Patients in Khartoum State Sudan. Nov Appro in Can Study. 4(1).NACS.000576..DOI: 10.31031/NACS.2019.04.00057

[26]. Ahmed HG, Suliman RSA , Abd El Aziz MS and Alshammari FD. (2015) Molecular screening for Epstein Barr virus (EBV) among Sudanese patients with nasopharyngeal carcinoma (NPC). Infectious Agents and Cancer 10:6 DOI 10.1186/s13027-015-0002-4,

[27]. Adam A, Nazik E, Lamyaa AM, Elwaleed M, Muntaser E, Ahmed M. (2014) detection of Epstein-Barr Virus in Nasopharyngeal Carcinoma in Sudanese by in Situ Hybridization.

[28]. Ahmed HG, Osman SI, Ashankyty IM. (2012) Incidence of Epstein-Barr virus in pediatric leukemia in the Sudan. Clin Lymphoma Myeloma Leuk.;12(2):127–31.

[29]. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 11;16(3):1215. doi: 10.1093/nar/16.3.1215.

[30]. Bornkamm GW. (2008-2009) Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: more questions than answers. Int J Cancer; 124(8):1745-1755.

[31]. Pietersma F, Piriou E, van Baarle D. Immune surveillance of EBV-infected B cells and the development of non-Hodgkin lymphomas in immunocompromised patients. Leukemia and Lymphoma; 49(6):1028-1041.

[32]. Johnston WT, Mutalima N, Sun D, Emmanuel B, Bhatia K, Aka P, Wu X, Borgstein E, Liomba GN, Kamiza S, Mkandawire N, Batumba M, Carpenter LM, Jaffe H, Molyneux EM, Goedert JJ, Soppet D, Newton R, Mbulaiteye SM. (2014) Relationship between Plasmodium falciparum malaria prevalence, genetic diversity and endemic Burkitt lymphoma in Malawi. Sci Rep; 4:3741. doi: 10.1038/srep03741.

[33]. Rasti,Kl Falk et al.(2005) circulating Epstein-Barr Virus in children living in malaria – endemic areas.scandinavian journal of immunology 61(5),461-465

[34]. Moormann AM, Snider CJ, Chelimo K. (2011) The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. Curr Opin Infect Dis; 24:435-441.

[35]. Chene A, Donati D, Guerreiro-Cacais AO, Levitsky V, Chen Q, et al. (2007) A molecular link between malaria and Epstein-Barr virus reactivation. PLoS Pathog., 3(6): e80. doi:10.1371/journal.ppat.003008

Cite this article as :

Khalid A. Abbas, Reham A. Alaidros, Salah aldeen Dahawi, Altayib Z. Mohammed, Awadalkareem Y. Awadalkareem, Mutaz A Elsir, Abdel Rahim M El Hussein, Isam M. Elkhidir, Khalid A. Enan, "Molecular Detection of Epstein Barr Virus in Malaria Positive Patients in Khartoum State Hospitals, Sudan 2018 ", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 9 Issue 6, pp. 317-322, November-December 2022. Available at doi : https://doi.org/10.32628/IJSRSET229650
Journal URL : https://ijsrset.com/IJSRSET229650