Vol. 13(2), pp. 13-18, July-December 2023
DOI: 10.5897/JMLD2023.0177
Article Number: 6E3D06771463
ISSN 2141-2618

http://www.academicjournals.org/JMLD

ACADEMIC JOURNALS
expand your knowledge

Journal of Medical Laboratory and Diagnosis

Full Length Research Paper

Correlation between severe malaria and EBV endemicity in children under five years in Ghana

Swabira Bashiru[1]*, Mina Ofosu[2], Alexander Martin-Odoom[3], Solomon Wireko[2] and Opoku Bempah[2]

[1]Department of Medical Laboratory Technology Royal Ann College of Health, Kumasi, Ghana.
[2]Department of Laboratory Technology, Kumasi Technical University, Kumasi, Ghana.
[3]Department of Medical Laboratory Sciences, University of Ghana, Kumasi, Ghana.

Received 8 August, 2023; Accepted 20 October, 2023

In Africa, *Plasmodium falciparum* accounts for majority of malaria morbidity and mortality in children under five years. At this age, they also become co-infected with Epstein-Barr virus (EBV). There is scanty of information whether this co-infection is the reason why malaria in children tends to be severe. The aim was to study the association between co-infection of EBV with *P. falciparum* infection and malaria severity in children under five. A cross sectional study was conducted among eighty children below five years who had been confirmed to have malaria. These children were grouped into those who had severe malaria and those who did not have severe malaria. EBV antibodies (EBNA-1 IgG) and malaria parasitaemia were measured from the blood samples of the children using ELISA and microscopy, respectively. EBV seroprevalence was higher in the severe malaria group, 16 (32.78%) compared to non-severe malaria group, 5 (16.1%); although it did not show any significant difference (Risk ratio: 2.024, 95% CI: 0.8249 to 4.9686, p = 0.1236). Despite the higher seroprevalence of EBV in the severe malaria group, this was not responsible for malaria severity. Children with EBV were however more likely to progress to severe malaria.

Key words: Epstein-Barr virus, *Plasmodium falciparum*, severe malaria, endemicity.

INTRODUCTION

Both Epstein-Barr virus (EBV) and *Plasmodium falciparum* infection have been proposed to be the etiological agents of endemic Burkitt's lymphoma (eBL). In co-infection, if any of the two pathogens disturb the immune system, it results in an imbalance between the host immunity and the pathogens ability to cause disease. This would eventually make the disease severe, which would have been otherwise if the pathogen were only one (Buttler et al., 2013; Pradhan and Gosh, 2013). Similarly, Griffiths et al. (2014) pointed out that, infections with more than one pathogen including malaria parasites are often linked with poor health outcomes due to higher parasite densities compared to those with single infection. Also, these co-infections result mostly in reduced treatment efficacies and increased cost of treatment.

In sub-Saharan Africa, most children become infected

*Corresponding author. E-mail: swabcuty@gmail.com. Tel: +233 546464965.

with EBV and undergo sero-conversion by the age of 3 years (Chene et al., 2009). It is within this age range that children also get infected with *P. falciparum* resulting in the increasing morbidity and mortality (Matar et al., 2015). In malaria endemic areas, these children are usually seropositive to EBV by six months of age which is in consonance with the decline of maternal antibodies (Piriou et al., 2012).

In Kenya for instance, EBV infected children residing in holoendemic areas have been demonstrated by Piriou et al. (2012) to have a poorly controlled viral infection; a hallmark of eBL. In another study by Chattopadhyay et al. (2013), exposure to holoendemic malaria has been associated with alteration in the differentiation of EBV-Specific $CD8^+$ T cell. Although much is known about the association of co-infection of EBV and *P. falciparum* with eBL (Haque et al., 2004), its association with severe malaria in children on the other hand has not gained that kind of popularity.

However, a breakthrough study which used a mouse model co-infected with murine herpes virus (MHV- 68) and *Plasmodium chabaudi* or *Plasmodium yoelii* XNL has provided an insight into how EBV influences antimalarial immunity. A significant similarity between MHV-68 and human gamma herpes virus has been established in terms of pathogenesis and biology (Doherty et al., 2001). Hence, MHV-68 has been used to show that gamma herpes virus can suppress the innate immunity to malaria thereby rendering a non-severe form of malaria to transform into a lethal one (Matar et al*.,* 2015).

The question of why some children recover from malaria while others do not need to be resolved. Therefore, this study was conducted to investigate the possible association between co-infection of EBV and the severity of *P. falciparum* malaria in children under five.

The present study provides a valuable insight to clinicians in the management of severe malaria. This would enable them to tackle EBV infections through the utilization and improvement of EBV antibody and therapeutic drugs against the virus in stable malaria region. This would give some sort of alleviation amid the improvement of severe form of malaria in these children.

MATERIALS AND METHODS

Study design and site

A cross sectional study was conducted among eighty children with malaria who were recruited from a Private Health Facility (PHF) and a Public Health Facility (PBHF) in the Western and Ashanti region, respectively. These children were further grouped into those who had severe malaria and those who did not have severe malaria (non-severe malaria). Those with non-severe malaria were used as a comparison group to detect whether any association existed between EBV and severe malaria.

Enrolment and grouping of the children

All children were recruited within a period of three months: from April to June. This period is marked as rainy season in Ghana. Any child brought to the laboratories in the two hospitals upon a clinician's request for blood film and met the inclusion criteria was eligible for the study. However, only children whose parents/guardian consented and signed the informed consent form to allow their children to participate in the study were enrolled.

Children confirmed with parasitaemia levels greater than 10,000 μl^{-1} and fever (core body temperature ≥37.5°C), diarrhoea, vomiting, malaise, pallor, prostration, convulsion, respiratory distress or any other symptoms were classified as having severe malaria (Squire, 2013). Children confirmed to have parasitaemia level less than 10,000 μl^{-1}, fever (core body temperature ≥37.5°C), diarrhea, vomiting, malaise, etc., were classified as having non-severe malaria (Squire, 2013).

Blood collection and processing

A minimum of 500 µl of venous blood was withdrawn from children under five years. The blood samples were put into EDTA tubes and tested for malaria, HIV and sickling status. Plasma was separated from red cells using a Hettich Universal 16A centrifuge at 1500 rpm for 5 min. It was then transferred into labelled Eppendorf tubes and stored at -20°C until further analysis. Children who tested positive to sickle test and HIV were excluded from this study.

Haematological analysis

Full blood count of whole blood samples was analysed using an automated haematology analyser (BC- 3000 Plus, Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China).

Thin film was used to identify *P. falciparum* whereas thick film was used to estimate parasite numbers. Sickling cell slide test and First Response HIV 1-2.0 rapid tests were used to determine the sickling and HIV status of the participants using method described in Cheesbrough (2005).

Identification of *P. falciparum* by thin film

Thin film was used in differentiating *P. falciparum* from other *Plasmodium* parasites.

Estimation of *P. falciparum* density by thick film

Thick film was used in the estimation of *P. falciparum* density in the blood. Parasites were counted against a pre-determined WBC of 200×10^9 L as described in Cheesbrough (2005).

Detection of Epstein-Barr Nuclear Antigen-1 (EBNA-1) IgG

EBV-specific immunoglobulin (IgG) to EBNA-1 was detected using EBV ELISA kits EIA-4246 following the manufacturer's protocol (DRG Instruments GMBH, Deutschland).

Statistical analysis

Analyses were carried out using IBM® SPSS® Statistics version 20. Continuous variables were compared with the t-test. Chi-square tests or Fisher's exact test were used for nominal variables. A *p* value of less than 0.05 was considered significant.

Ethical considerations

Approval of this study was obtained from the Ethics and Protocol

Review Committee of the School of Biomedical and Allied Health Sciences, College of Health Sciences. The Ethics Identification number is SBAHS-MD/10551998/AA/5A/2016-2017.

RESULTS

The mean age, WBC, parasite density, core body temperature and percentage of gender, age group, EBV seropositive and the use of insecticide-treated net has been summarized in Table 1.

There was no significant difference in age and gender between severe malaria and non-severe malaria group. However, a significant difference was found in WBC, duration of illness, core body temperature (all Ps <0.05). The objective of the study was to determine if EBV was associated with malaria severity in children less than five years, hence the need to determine their malaria status. In addition, the presence of EBV was determined in the plasma of these children to know if EBV was associated with malaria severity. A significant difference was found in parasite density between the two malaria groups (P<0.05). Those classified as severe malaria had higher parasitaemia than the non-severe malaria group (Figure 1). Figure 2 shows the prevalence of EBV among the severe and non-severe malaria group.

Out of the 31 non-severe malaria group, 5(16.1%) children were seropositive to EBV whereas 26 (83.9%) children were seronegative. In the severe malaria group, 16 (32.78%) children were seropositive to EBV whilst 33 (67.3%) children were seronegative to EBV. Of note, those with severe malaria showed higher prevalence of EBV than the non-severe group; however, no significant difference existed between the two.

DISCUSSION

There is robust evidence that, EBV is essential but it cannot on its own suffice to cause eBL in children. However, it can cause eBL only if, it is associated with a cofactor like *P. falciparum. P. falciparum* destroys immunity to EBV thereby resulting in a large number of B cells being infected with EBV, a hallmark of Burkitt's lymphoma (Moormann et al., 2011). The cofactor, *P. falciparum* has been proposed to be a bad company to EBV in the pathogenesis of eBL. However, only a limited research has been undertaken to otherwise find out if EBV can also be a bad company to *P. falciparum* in the pathogenesis of severe malaria. This study therefore, sought to find out whether there was also an association between co-infection of EBV and *P. falciparum* with malaria severity in children under five.

The current study showed more males than females; however, the difference was not statistically significant. A study in Uganda by Danielle and Glenda (2016) also found a similar observation. This implied that, gender might not be a risk factor for malaria. According to UNDP (2015), malaria is not gender bias and this explains why gender was not a risk factor for malaria in this study. The absence of any statistical difference in age between severe and non-severe malaria group made it easier to make comparison between them without any bias.

Humoral immune response to malaria is one of the effective means of downregulating the level of parasitaemia in humans and this serves as a form of protective immunity (Langhorne et al., 2008). In malaria infection, changes in haematological features such as white blood cells (WBC) are known to occur. WBC count varies during every stage of malaria infection. Leucopenia for instance, is associated with acute malaria whereas leucocytosis occurs in severe malaria. Alterations in WBC count have been linked to severity of infection and malaria is no exception (WWARN, 2019).

WBC has been found to be higher in patients with high parasitaemia compared to those with low and moderate parasitaemia (Kotepui et al., 2015). Similarly, to the aforementioned findings, there was a marked difference in white blood cell counts between severe and non-severe malaria group.

The possible reason could be because of the higherparasitaemia in the severe malaria group compared to the non-severe group. This finding is in consonance with the report of Mckenzie et al. (2005), Abro et al. (2008) and Simji et al. (2017).

In this study, EBNA-1 IgG was detected in the plasma of both groups of children irrespective of being diagnosed as severe or non-severe malaria. This implied that, EBV can infect children at a younger age as reported by other studies (Piriou et al., 2012). A lower age-specific prevalence of EBV antibodies was found in this study as compared to that which was found among children in Thailand (Rabporn et al., 2015). This was probably due to the fact that, only EBNA-1 IgG was used as a marker of EBV infection in this study. In the Thailand children however, anti-VCA IgG was used as a marker of EBV infection. Anti-VCA IgG is a marker of recent infection whilst Anti-EBNA-1 IgG is a marker of past infection (Paschale and Clerici, 2012). Besides, the prevalence in this study was specific to children presented with only malaria. The absence of EBV infection in the 0 to 7 months age group could be attributed to the presence of maternal antibodies. A study by Piriou et al. (2012) reported that, martenal antibodies in newly born children prevent them from EBV infection. EBV infection can only occur after the waning of maternal antibodies.

Primary infection with EBV has been found to damage the production of malaria specific antibodies against plasmodium parasites. This is because EBV infects B cells which are known to produce antibodies against malaria. These infected B cells become incompetent in fighting the malaria parasite and this renders a non-severe malaria infection to become severe and probably lethal (Matar et al*.,* 2015). The need therefore, to determine whether EBV has a causal association with a

Table 1. The demographic characteristics and clinical information of the two malaria groups (severe and non-severe).

Category	Variable	Malaria group		P-value
		Non-severe (N=31)	Severe (N=49)	
Age in months (Mean ± SEM)		27.61±2.652	25.76±2.101	0.5842
Age group (months)	1-7	1(33.3)	2(66.7)	0.8896
	8-23	10(35.7)	18(64.3)	
	24-48	20(40.8)	29(59.2)	
Gender	Male	15(31.9)	32(68.1)	0.1650
	Female	16(48.5)	17(51.5)	
Use of insecticide-treated net (itn)	Yes	21(40.45)	31(59.6)	0.0819
	No	10(35.7)	18(64.3)	
Core body temperature (ºC)		37.55±0.1503[a]	38.4±0.0.09184[a]	0.0001*
Duration of illness (days)		1.613±0.2004[a]	2.286±0.2278[a]	0.0436*
Parasite density (/µl)		2009±462.6	43293±3718	<0.0001*
WBC (10^9/L)		7.919±1.299	12.24±1.066	0.0126*
EBV seropositive		5(16.1)	16(32.78)	0.1236

*Significant at <0.05; [a]Mean ± SEM. The figures in parenthesis represent percentage (%).

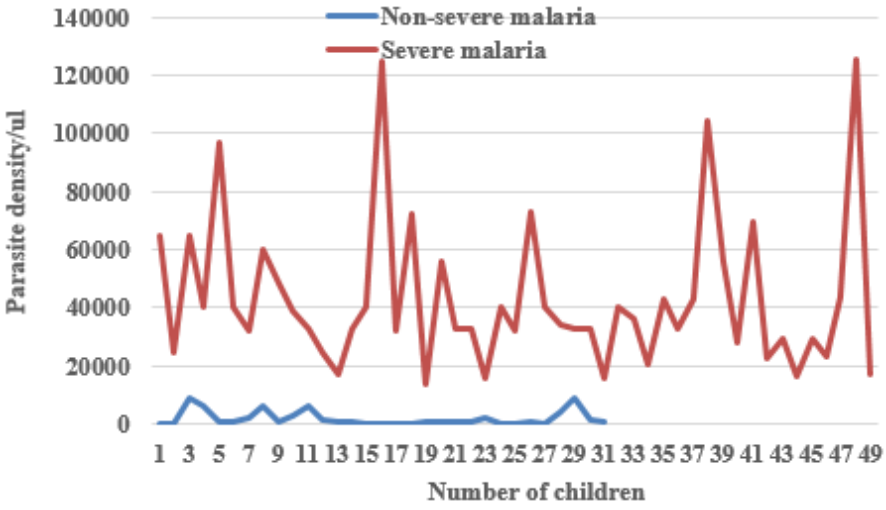

Figure 1. Parasite density levels between the two groups of children.

specific disease or it is just a passer-by (Paschale and Clerici, 2012).

The study however, did not find any significant association between EBV serostatus and the degree of malaria. This implied that, co-infection of EBV and *P. falciparum* in children with malaria was not linked to severity of malaria in this study. There was also no association between gender and EBV serostatus even though the number of males who seroconverted was more than females. Hence, gender might not be a risk factor in acquisition of EBV infection. This finding agrees with other studies (Haque et al., 2004; Sidorchuk et al., 2003; Adjei et al., 2008; Balfour et al., 2013).

Infants were likely to have gotten infected either by saliva on toys, fingers, the act of pre-chewing of foods by their mothers, organ transplant or breast milk (Hoover and Higgnibotham, 2023; Ibrahim et al., 2015). These modes of transmission are also not gender bias and this might have accounted for the lack of association between EBV and gender. Male seropositive rate being higher than female seropositive rate can also be attributed to the fact that, there were more males than females in the study.

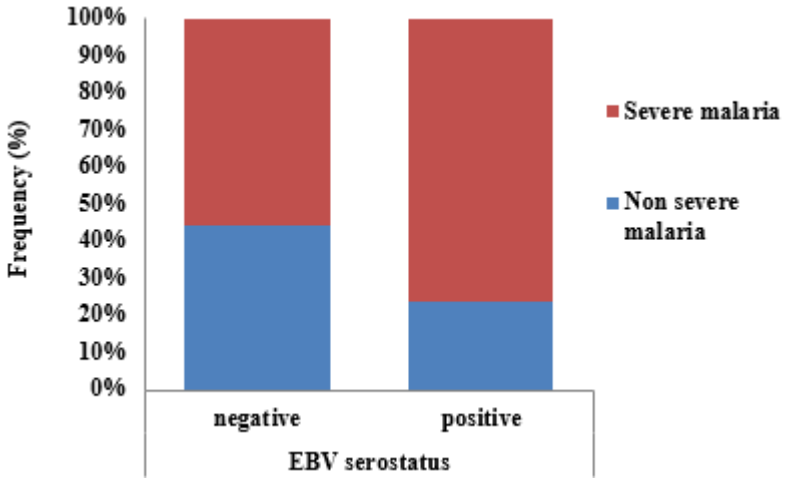

Figure 2. Distribution of EBV between the two malaria groups.

The lack of significant association between co-infection of EBV and *P. falciparum* in this study might be because only the EBNA-1 was targeted. This was also parallel to the findings of Matar et al. (2015) who used a mouse model to elucidate the impact of this co-infection on the severity malaria. The study found that, latent EBV infection was not associated with severe malaria.

Conclusion

The study determined that, despite children with severe malaria having a higher seroprevalence of EBNA-1 IgG, this was not the sole factor responsible for malaria severity. This is because children with non-severe malaria also had EBNA-1 IgG present in their blood plasma. However, it was observed that children with EBV were more likely to progress to severe malaria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors express their gratitude to the staff of both health facilities concerned for unflinching support during this study.

REFERENCES

Abro AH, Abdalla MU, Nadeem JY, Ahmed AS, Dujana A, Ahmed AS (2008). Malaria and haematological changes. Pakistani Journal of Medical Sciences 24(2):287-291.

Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, Asare I (2008). Seroprevalence of HHV-8, CMV and EBV among the general population in Ghana, West Africa. BMC Infectious Disease 8:111.

Balfour Jr HH, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W (2013). Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. The Journal of infectious Diseases 208(8):1286-1293.

Buttler NS, Harris TH, Blader IJ (2013). Regulation of immunopathogenesis during Plasmodium and Toxoplasma infections: more parallels than distinctions? Trends in Parasitology 29(12):593-602.

Chattopadhyay PK, Chelimo K, Embury PB, Mulama DH, Sumba PO, Gostick E, Ladell K, Brodie MT, Valule J, Roederer M, Moorman AM, Price DA (2013). Holoendemic malaria exposure is associated with altered Epstein-Barr virus-specific CD8+ T-cell differentiation. Journal of Virology 87(3):1779-17788.

Cheesbrough (2005). District laboratory practice in tropical countries (Part 1). Cambridge: Cambridge University Press pp. 246-335.

Chene A, Daria D, Jackson O, Mbidde ER, Fred K, Wahlgren M, Bejarano MT (2009). Endemic Burkitt's lymphoma as a polymicrobial disease: New insights on the interaction between Plasmodium falciparum and Epstein-Barr virus. Seminars in Cancer Biology 19(6):411-420.

Danielle R, Glenda M (2016). Risk factors of malaria in children under the age of five years old in Uganda. Malaria Journal 15(246).

Doherty PC, Christensen JP, Belz GT, Stevenson PG, Sangster MY (2001). Dissecting the host response to a gamma-herpesvirus. Philosophical Transactions of the Royal Society of London B: Biological Science 356(1408):581-593.

Griffiths EC, Pedersen AB, Fenton A, Petchey OL (2014). Analysis of a summary network of co-infection with Humans reveals that parasites interact most via shared sources. Proceedings of the Royal Society B- Biological Sciences 281(1782):20132286.

Haque A, Rachinel N, Quddus MR, Haque S, Kasper LH, Usherwood E (2004). Co-infection of malaria and γ-herpesvirus: exacerbated lung inflammation or cross-protection depends on the stage of viral infection. Clinical Experimental Immunology 138(3):396-404.

Hoover K, Higginbotham K (2023). Epstein barr virus. In StatPearls (internet), Treasure Island (FL): StatPearls publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK559285/.

Ibrahim ID (2015). Effect of *Plasmodium falciparum* malaria on Epstein-BarrVirus(EBV). Reactivation during pregnancy and subsequent shedding of EBV in breast milk postpartum. Maternal Child Health Journal. 19(3):606-614.

Kotepui M, Piwkham D, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S (2015). Effect of malaria parasite density on blood cell parameters. Plos ONE 10(3):e01121057

Langhorne J, Francis MN, Anne-Marit S, Kevin M (2008). Immunity to

malaria: more questions than answers. Nature Immunology 9(7):725-732.
Matar CG, Anthony NR, O'Flaherty BM., Jacobs NT, Priyamvada L, Engwerda C.R, Speck SH, Lamb TJ (2015). Gammaherpesvirus co-infection with malaria suppresses anti-parasitic humoral immunity. Plos Pathogens 11(5):e1004858.
McKenzie FE, Prudhomme WA, Magill AJ, Forney JR, Permpanich B, Lucas C, Robert A, Gasser RA, Wongsrichanlai C (2005). White blood cell counts and malaria. The Journal of infectious diseases 192(2):323-330.
Moormann AM, Cynthia JS, Kiprotich C (2011). The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. Current Opinion in Infectious Disease 24(5):435-441.
Paschale M, Clerici P (2012). Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. World Journal of Virology 1(1):31-43.
Piriou E, Amolo SA, Sumba O, Fiore N, Middeldorp JM, Moormann AM, Ploutz-Synder R, Rochford R (2012). Early age at time of primary Epstein-Barr virus infection results in poorly controlled viral infection in infants from Western Kenya: Clues to the etiology of endemic burkitt lymphoma. The Journal of Infectious Diseases 205(6):906-913.
Pradhan V, Gosh K (2013). Immunological disturbances associated with malaria infection. Journal of Parasitic Diseases 37(1):11-15.
Rabporn S, Nasamon W, Sompong V, Thanunrat T, Jira C, Yong P (2015). Seroprevalence of anti-EBV IgG among various age groups from Khon Kaen province, Thailand. Asian Pacific Journal of Cancer Prevention 16(17):7583-7587.
Sidorchuk A, Lagarde F, Pershagen G, Wickman M, Linde A (2003). Epstein-Barr virus infection is not associated with development of allergy in children. Pediatric Infectious Diseases Journal 22(7):642-647.
Squire DS (2013). Malaria parasitaemia levels in relation to antioxidant enzyme levels in severe malaria amongst Ghanaian children. Retrieved from: http://ugspace.ug.edu.gh
Simji SG, Akingdih MT, Ibrahim HO, Isa SE, Anejo-Okopi J (2017). Prevalence of malaria and its association with ABO blood group in Jos, Nigeria. International Journal of Infectious Diseases and Therapy 2(3):59-65.
United Nations Development Programme (UNDP) (2015). Discussion Paper: Gender and malaria. Available at: https://www.undp.org
WWARN (2019). Study group plans to identify what determines white blood cell count during malaria infection. Available at: http:www.wwarn.org.new/newsl/articles.