

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

**CORRELATION BETWEEN EPSTEIN-BARR VIRUS INFECTION
AND THE SEVERITY OF MALARIA IN GHANAIAN CHILDREN
UNDER FIVE (5) YEARS OF AGE**

BY

SWABIRA BASHIRU

(10551998)

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
MSC MEDICAL LABORATORY SCIENCE DEGREE**

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

JULY 2017

DECLARATION

I, Swabira Bashiru, hereby declare that, except for reference to other people's work, which I have duly cited, this thesis is the result of an original research work and that the material has not been presented either in whole or in part elsewhere for another degree and all experimental works was performed by me under the supervision of Dr. Alexander Martin-Odoom and Dr. Charles Brown.

Swabira Bashiru
(Student)

Date

Dr. Alexander Martin-Odoom
(Supervisor)

Date

Dr. Charles Brown
(Supervisor)

Date

ABSTRACT

Background: Majority of malaria morbidity and mortality mostly arise in children younger than five years and this also happens to be the same period of time they become co-infected with Epstein –Barr virus (EBV). In areas of endemic malaria transmission, immunity to non-cerebral severe malaria is expected to be acquired after 1-2 infections with *Plasmodium falciparum*. Despite this immunity, 20% infected children die yearly due to severe malaria. Previous studies have shown that, dual infection of *P. falciparum* and EBV is the cause of endemic Burkitt's lymphoma (eBL). However, there is scanty of information whether this same co-infection is the reason why malaria in children tends to be severe.

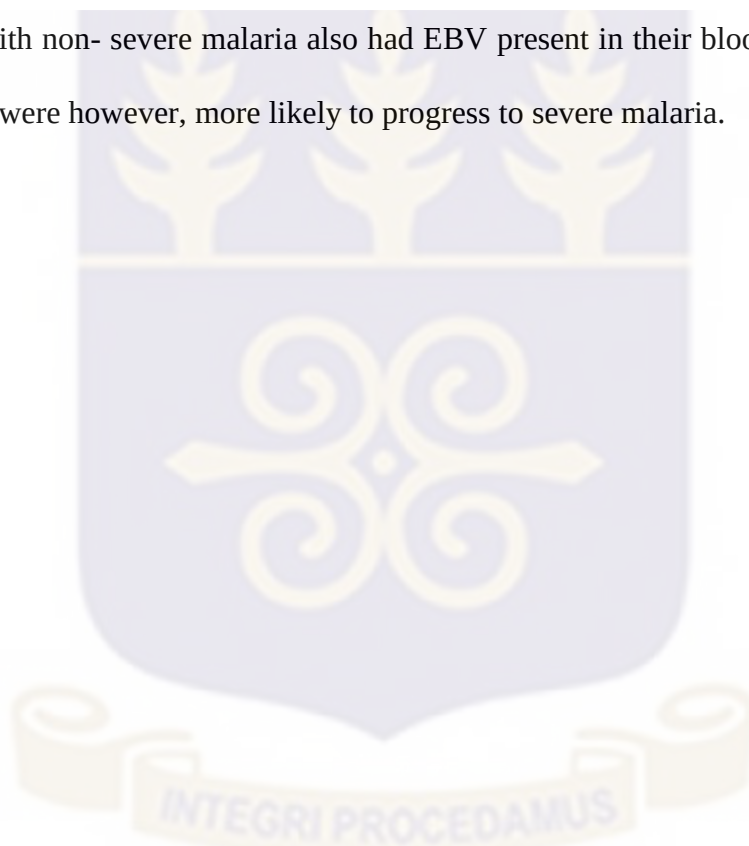
Aim: The aim was to study the association between co-infection of EBV with *P. falciparum* infection and malaria severity in children under five years of age.

Method: The study population comprised of children below 5 years of age who had been clinically diagnosed and confirmed to have malaria at the Divine Love and Nkenkensu Government hospitals. The study design was a cross sectional study and the convenient sampling technique was used to enrol 80 children with malaria. These children were then classified into two groups; severe malaria and non-severe malaria. ELISA was used to detect EBV specific immunoglobulins to EBNA-1 EBV antigen in the plasma of these children.

Results: In all, there were 49 (61.25%) children classified as having severe malaria and 31 (38.75%) children with non-severe malaria. Parasite density levels between the two groups showed a clear separation. WBC and parasite density also showed statistically significant differences (both $p < 0.05$), compared to both RBC and Hb concentrations (both $p > 0.05$) between the two groups. Clinical histories of the two malaria groups for body temperature, duration of illness, fever and malaise, showed strong statistically

significant differences (all $p \leq 0.0001$). The overall seroprevalence of EBV in this study was 26.25%. The seroprevalence of EBNA-1 in children classified as severe malaria was 32.78%. No EBNA-1 IgG was found in children between 0-7 months. There was no significant association ($p = 0.123$) between severe malaria and EBV infection though children with EBV were more likely to develop severe malaria (Risk ratio 2.024, 95% CI 0.8249 to 4.9686, $p = 0.1236$) .

Conclusion: The study determined that even though children with severe malaria had higher seroprevalence of EBV, this was not responsible for malaria severity since children with non- severe malaria also had EBV present in their blood plasma. Children with EBV were however, more likely to progress to severe malaria.



DEDICATION

This work is dedicated to Almighty God,
my parents Ahaji Bashiru Faaza and Hajia Ayisha Abdullai, my mentor, Mma Asana
and to my supervisors, Dr Martin-Odoom and Dr. Charles Brown.
Your effort has been immensely appreciated.



ACKNOWLEDGEMENTS

First and foremost, I give thanks to almighty God for giving me the gift of life. My profound appreciation also goes to my supervisors, Dr. Alexander Martin-Odoom and Dr. Charles Brown, for their immense contribution in terms of guidance, support and the provision of all the materials needed for this study. To, Dr Charles Brown again, I am immensely grateful to him for the extra trouble he undertook to make sure my write-up was “okay”

I am forever thankful to my parents, especially my father for giving me another chance of education. You have been my strength and the reason I have come this far, thank you once again. To Mma Asana, you have been with me all this while to. I thank for your support in diverse ways.

I will also not hesitate to thank the Staff of Divine Love hospital and Nkenkenu Government hospital for making me use their facility. I am highly indebted to the laboratory staff of these two hospitals for assisting me in the sample collection and laboratory work. I am so much indebted to John Justice Obeng Manu for his support throughout my stay in Bibiani during the sampling period. To Mr. Boamah of Virology department, School of Biomedical and Allied Health Sciences and Mr Alabira of Lapaz Community hospital, I am so much grateful for your patience and assistance you offered during the course of running the ELISA.

To all 2015 MSc. Medical laboratory science students, I thank you all for the support and encouragement you all gave in times of difficulties.

TABLE OF CONTENTS

DECLARATION	ii
ABSTRACT.....	iii
DEDICATION.....	v
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES	x
LIST OF TABLES.....	xi
LIST OF ABBREVIATIONS.....	xii
CHAPTER ONE	1
INTRODUCTION	1
1.1 BACKGROUND.....	1
1.2 PROBLEM STATEMENT	3
1.3 SIGNIFICANCE OF STUDY.....	4
1.4 AIM.....	5
1.5 SPECIFIC OBJECTIVES	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 EPSTEIN-BARR VIRUS (EBV).....	6
2.1.1 Biology	6
2.1.2 Epidemiology	6
2.1.2.1 Age of primary infection.....	8
2.1.2.2 Sero-epidemiology.....	10
2.1.3 Transmission.....	12
2.3.4 Host Range and Target Site	13
2.1.4 Pathogenesis	14
2.2 MALARIA	17
2.2.1 History	17

2.2.2 Epidemiology	19
2.2.2.1 Transmission.....	19
2.2.3 Life Cycle of <i>Plasmodium</i> Parasites.....	20
2.2.4 Symptoms	22
2.2.5 Humoral Immune Response	23
2.2.6 Cell-mediated Immunity.....	25
2.2.7 Immunity to Malaria in Infants.....	25
2.2.8 Severe Malaria.....	26
2.3 CO-INFECTION OF EPSTEIN- BARR VIRUS AND <i>PLASMODIUM FALCIPARUM</i>	27
2.3.1 Gamma Herpes Virus and Malaria Immunity	28
CHAPTER THREE	32
MATERIALS AND METHODS.....	32
3.1 STUDY DESIGN.....	32
3.2 STUDY SITES.....	32
3.3 STUDY POPULATION AND SAMPLING METHOD	33
3.3.1 Inclusion Criteria	33
3.3.2 Exclusion Criteria.....	33
3.5 SAMPLE SIZE CALCULATION	34
3.6 ENROLMENT AND GROUPING OF THE CHILDREN.....	34
3.7 HAEMATOLOGICAL AND PARASITOLOGICAL ANALYSIS.....	35
3.7.1 Blood Collection and Processing.....	35
3.7.2 Haematological Analysis.....	35
3.7.2.1 Sickling test.....	35
3.7.2.2 Identification of <i>P. falciparum</i> by thin film	36
3.7.2.3 Estimation of <i>P. falciparum</i> density by thick film	36

3.7.2.4 Detection of HIV antibodies	37
3.7.3 Detection of Epstein-Barr Nuclear Antigen-1 (EBNA-1) IgG	37
3.7.3.1 Assay procedure.....	37
3.7.3.2 Measurement.....	38
3.7.3.3 Results.....	38
3.7.3.3.1 Validation.....	38
3.7.3.3.1 Calculation	38
3.7.3.3.1 Interpretation.....	39
3.8 STATISTICAL ANALYSIS.....	39
3.9 ETHICAL CONSIDERATIONS	39
CHAPTER FOUR.....	41
RESULTS	41
4.1 DEMOGRAPHIC DATA OF THE CHILDREN	41
4.2 CLINICAL HISTORY	43
4.3 HAEMATOLOGICAL ANALYSES	43
4.4 DETECTION OF EPSTEIN-BARR NUCLEAR ANTIGEN-1 (EBNA-1) IgG...	46
4.5 CORRELATION BETWEEN EBV AND BOTH CLINICAL HISTORIES AND HAEMATOLOGICAL PROFILES OF THE TWO GROUPS OF CHILDREN.....	47
4.6 ASSOCIATION BETWEEN THE SEVERE MALARIA GROUP AND EBV ...	48
CHAPTER FIVE	49
DISCUSSION AND CONCLUSIONS	49
5.1 DISCUSSION	49
5.2 CONCLUSIONS.....	53
REFERENCES	55
APPENDIXES	70

LIST OF FIGURES

Figure 1	Pathogenesis of EBV infection.	15
Figure 2	Diagrammatic representation of the life cycle of <i>Plasmodium</i> parasites.	22
Figure 3	A model of how EBV interferes with malaria Immunity.	30
Figure 4	Parasite density levels between the two groups of children.	45
Figure 5	Distribution of EBV among the two malaria groups.	46

....



LIST OF TABLES

Table 1	Demographic information of the two groups of children	42
Table 2	Clinical histories of the two groups of children	44
Table 3	Haematological parameters of the two groups of children	45
Table 4	Correlation between EBV and haematological profiles of the children	47
Table 5	Correlation between EBV and clinical histories of the children	47



LIST OF ABBREVIATIONS

APC	Antigen presenting cell
CIDR1 α	Cysteine-rich interdomain region 1 α
CTL	Cytotoxic lymphocytes
DLH	Divine Love Hospital
DNA	Deoxyribonucleic acid
EA	Early Antigen
eBL	Endemic Burkitt's lymphoma
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
HIV	Human immune deficiency virus
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
iRBC	Infected red blood cell
LCMV	Lymphocytic Choriomeningitis virus
NGH	Nkenkensu Government Hospital
NK	Natural killer
PfEM1	<i>Plasmodium falciparum</i> erythrocyte membrane 1
TGF	Tumour growth factor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
VCA	Viral capsid antigen

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Six species of malaria parasites belonging to the family Plasmodiidae, of the Phylum Apicomplexa are the known cause of human malaria. These are *Plasmodium vivax*, *P. malariae*, *P. ovale curtsi*, *P. ovale wallikeri*, *P. falciparum* and *P. knowlesi* (Warrell *et al.*, 2012; Poirier *et al.*, 2016). However, the major cause of malaria in humans is *P. falciparum* (WHO, 2016). According to the report released by WHO in December 2016, there were 242 million malaria cases in 2015 and also 429,000 deaths (WHO, 2016). Sub-Saharan Africa still carries the highest portion of the global malaria burden with 90% of malaria cases and 92% fatality rate as at 2016. In areas of high malaria transmission intensity, young children at the age of 5 years and below are more susceptible to this infection. More than 70% of malaria associated deaths occurs within this age range (WHO, 2016).

Malaria infection is categorized into uncomplicated and severe (complicated) malaria (CDC, 2015). In uncomplicated malaria, the clinical presentation of the disease includes: headache, chills and diaphoresis. Other common symptoms are dizziness, mild diarrhoea, nausea, vomiting and dry cough (Genton & D'Acremont, 2001). Timely and proper treatment of uncomplicated malaria is very crucial in preventing its progression into severe disease and consequently death.

Severe malaria accounts for approximately one million mortalities yearly with children under the age of 5 years being the most susceptible of all (WHO, 2012). Severe malaria ensues when there are complications in malaria infections as a result of dysfunction of

vital organs or abnormalities in blood components or metabolism (CDC, 2015). Presentation of severe malaria is characterised by; cerebral malaria, low haemoglobin level, blood in urine, pulmonary oedema, defect in blood coagulation, low blood pressure, acute kidney failure, hyperparasitaemia, metabolic acidosis and low glucose concentration (CDC, 2015). However, the clinical indication of severe malaria in children under 5 years in Sub-Saharan Africa is characterised by two main outcomes; cerebral malaria and severe anaemia (WHO, 2014). Other manifestations such as cough, diarrhoea, and vomiting associated with malaria have also been documented (Anumudu *et al.*, 2004).

Multifactorial events such as high parasitaemia, longer period of illness prior exposure to antimalarial treatment or absence of protective interventions against mosquito bites have been linked to increasing risk of acquiring severe form of malaria (Byakika-Kibwika, 2009). However, it is yet to understand clearly why infection with malaria becomes severe in children below 5 years of age and eventually leads to death. Co-infection with another pathogen could also be a contributory factor to severity of malaria in a host (Haque *et al.*, 2004).

One such pathogen is Epstein-Barr virus (EBV) otherwise, known as human gamma herpes virus 4 (HHV4). Epstein-Barr-virus is oncogenic by nature and persists for life in its host (man). It affects greater than 90% of people worldwide (Chu-Qiong, & Jiman, 2016). In Ghana, Rasti *et al.* (2005) reported that, 47% of Ghanaian children with malaria had EBV DNA in their circulation. Epstein-Barr virus clinically manifests as a subclinical infection or acute mononucleosis (Silins *et al.*, 2001; Young & Rickinson, 2004). The asymptomatic infection is dominant during childhood after primary infection

but if it occurs during adolescence or later in life, the result is acute mononucleosis (Jayasooriya *et al.*, 2015). Epstein-Barr virus has two replication cycles: the latent and lytic cycle. The former persists in B cells and the latter occurs when there is reactivation of the latent form which consequently leads to it (lytic form) being shed into the saliva (Rickinson & Kieff, 2001).

Epstein- Barr virus causes many cancers which include B cell lymphoma, nasopharyngeal carcinoma and endemic Burkitt's lymphoma (eBL) amongst immunocompromised hosts (Thompson & Kurzrock 2004). In equatorial Africa, the childhood cancer, eBL occurs if EBV infection in children remains untreated for a longer period (Piriou *et al.*, 2012). Co-infection of EBV with holoendemic malaria is a potential risk factor of eBL (Rainey *et al.*, 2007; Jeanette *et al.*, 2008; Chattopadhyay *et al.*, 2013). Epstein-Barr-virus and *P. falciparum* are two pathogens whose infection can be modulated independently of each other but co-infection can probably become more lethal than each individual infection (Matar *et al.*, 2015).

1.2 PROBLEM STATEMENT

Plasmodium falciparum still remains the commonest amongst the four human malaria parasites in Ghana. Malaria mortality is concentrated among those who are immunocompromised and generally children below 5 years bear the brunt of the burden of malaria mortality (WHO, 2016). Children born into *P. falciparum* endemic areas usually acquire and clear the infections without showing any symptoms of illness, however, majority of them at some point in their lives will show symptoms of the infection which is presented as fever. These clinical responses may result in severe forms of complications and may result in severe complications and may heal naturally,

need medical intervention or better still leads to death (Robert & Omumbo, 2006). Although much is known about the association of co-infection of EBV and *P. falciparum* with eBL (Haque *et al.*, 2004), little is known about its association with severe malaria in children

As to why some children recover from malaria whilst others do not is a question which needs to be answered. Hence, the need for finding the possible association between co-infection of EBV and severity of *falciparum* malaria in children as well as determining the prevalence of EBV among children under 5 years of age who have been diagnosed of severe malaria.

1.3 SIGNIFICANCE OF STUDY

Children in sub-Saharan Africa mostly become co-infected with EBV and *P. falciparum*. EBV maintains a latent infection and then persists throughout the life of its host (Speck & Ganem, 2010). In malaria endemic areas these children are usually seropositive to EBV by 6 months of age which is in consonance with the decline of maternal antibodies (Piriou *et al.*, 2012). Almost all infected children would have undergone sero-conversion by age of 3 years (Arnaud *et al.*, 2009). It is within this age range that children get infected with *P. falciparum* resulting in the increasing disease and death among these children (Matar *et al.*, 2015).

Findings of the research would provide a valuable insight to clinicians in the management of severe malaria so as 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.

1.4 AIM

The research determined the correlation between co-infection of EBV and *P. falciparum* with the severity of malaria in children below 5 years old.

1.5 SPECIFIC OBJECTIVES

The specific objectives were to:

1. determine the overall seroprevalence of EBNA-1 IgG in the children with malaria as well as the seroprevalence of the children classified as having severe malaria.
2. compare the clinical histories and haematological profiles of children classified as having severe malaria to those classified as non-severe malaria.
3. establish any correlation between EBV and both clinical histories and haematological profiles of the children classified as having severe malaria to those classified as non- severe malaria.
4. establish any association between the severe malaria group and EBV

CHAPTER TWO

LITERATURE REVIEW

2.1 EPSTEIN-BARR VIRUS (EBV)

2.1.1 Biology

Epstein- Barr virus, originally named as the human herpes virus 4 (HHV-4) is an enveloped virus composed of 184-kbp double-stranded, linear deoxyribonucleic acid (DNA) genome that codes for more than 85 genes (Rickinson & Kieff, 2001). It is bounded by a protein capsid and in between this capsid and envelope is the tegument. On the outer envelope of the virus are glycoproteins that are essential for targeting specific cells, host species and for communication with the host cell via ligand-receptor pathway (Rickinson & Kieff, 2001). The genome is made up of a series 0.5kb repeats which are terminally arranged in tandem and have internally repeated sequences that partitions the genome into long and short distinct regions that have majority of the coding capability (Thompson & Kurzrock, 2004).

In humans, there are two recognized types of EBV; EBV-1 and EBV-2 also referred to as Type A and B. These two vary in the gene sequence that encodes the EBV nuclear antigens; EBNA-2, EBNA-3A/3, EBNA-3B/4 and EBNA-3C/6 (Sample *et al.*, 1990).

2.1.2 Epidemiology

Epstein-Barr virus is widely spread and infects almost all population sub-clinically during childhood and thereafter: maintains a life- long persistence in its host. The virus establishes itself in antibody-producing memory B cells which serve as the ideal environment for its long-term residence (Crawford, 2001). According to the World Health Organisation International Agency for Research of Cancer (IARC), EBV is

categorized under group 1 carcinogen and it is known to directly have a role in the pathogenesis of eBL, nasopharyngeal carcinoma, gastric carcinoma and a subclass of Hodgkin lymphoma (IARC, 1997). The childhood cancer, Burkitt's lymphoma which is characterised by jaw tumour with multiple quadrants is chiefly caused by the Co-infection of EBV and *P. falciparum* (Mutalima *et al.*, 2008). Not less than 90% of eBL cases have been proven to be EBV-associated with supportive corroborations such as the existence of EBV-DNA clonally integrated in tumour tissue and sero-epidemiological associations with EBV antibodies (Sugden, 2014).

Currently, there are two distinct types of EBV isolates which have been identified. These isolates were originally classified as A and B but presently designated as type 1 and 2 (IARC, 1997). EBV-1 and EBV-2 do not only differ in their sequence of genes that encodes EBV nuclear antigen (EBNA) (Buisson *et al.*, 1994) but also in their geographical distribution. They are both found all over the world but with different geographical distribution. Although the EBV-1 is more predominant than EBV-2, in some regions including Western New Guinea, Central Africa and north-western part of Canada, EBV-2 is more common (Kwok *et al.*, 2015). In equatorial Africa for instance where holoendemic malaria and Endemic Burkitt's lymphoma are very common about half of Endemic Burkitt's lymphomas have been proven to carry EBV-2. However, in Taiwan, more than half of nasopharyngeal carcinomas carry EBV-1 (Shu *et al.*, 1992).

The underlying mechanism associating infection of EBV with the development of malignant B cells leading to jaw tumour is unknown. There seems however to be two compelling theories even though totally unrelated to elucidate the association between EBV and *P. falciparum* as the cause of eBL (Rochford *et al.*, 2005). One theory

proposes that, *P. falciparum* activates the proliferation of polyclonal B cell and lytic EBV reactivation that leads to increase in numbers of latently infected B cells and the propensity of *c-myc* translocation; a hallmark of eBL. The second theory suggests that, EBV-specific T-cell immunity is disrupted in the course of *P. falciparum* co-infection due to enhanced B cell replication thus leading to loss of viral control (Moormann *et al.*, 2011).

Primary infection with EBV have been found to damage the production of malaria-specific antibodies derived from the humoral immune response that control plasmodium parasitaemia thereby rendering a non-severe malaria infection to become severe and probably lethal (Matar *et al.*, 2015)

2.1.2.1 Age of primary infection

The age at which primary EBV infection has remained a debatable one among researchers as others argue that EBV infection cannot occur earlier in the lives of children whereas others uphold the fact that primary EBV infection can actually occur in their early life. Among those researchers of the former hypothesis are Biggar *et al.* (1978) who hypothesized that, infection of EBV at a younger age is not possible because infants have maternal antibodies circulating in their blood and this serves as immunity to various infections and EBV is no exception. According to Biggar *et al.* (1978), EBV infection in children can occur only after waning of maternal antibodies. This finding is also parallel to the findings of Chan *et al.* (2001) who also proposed that primary infection with EBV among infants below six (6) months old is not possible.

Contrary to the findings of these researchers, de Thé *et al.* (1978) hypothesized that infection of children at an earlier age is possible and may lead to poor control of the virus by the host and this serve as a risk factor of eBL. Additionally, it has also been proposed that, infection with EBV mostly arises in individuals at a younger age in developing countries due to low socio-economic status and poor hygienic conditions (IARC, 1997). In Africa for instance, most children become infected early in their lives and by three (3) years of age, antibodies of EBV are known to circulate in the serum or plasma of most of them. However, primary EBV infection in developed countries is known to delay till adolescence (Hjalgrim *et al.*, 2007).

EBV infection has also been hypothesized by Slyker *et al.* (2013) to occur earlier in the life of children of HIV-infected mothers as compared to their counterpart born to HIV-negative mothers. Malaria transmission intensity according to Priou *et al.* (2012) is also a contributory factor by which children acquire EBV earlier in their life. In this work, two cohorts of infants in two areas with divergent malaria exposure were being followed. Results from the work showed that infants from Kisumu (stable /high malaria transmission region) were significantly infected with EBV at younger age compared to children in Nandi (unstable malaria transmission). Rasti *et al.* (2005) in their work also found greater percentage (47%) of EBV in the plasma of children in Ghana (aged between 5months and twelve years) and 34% amongst non-malaria group but was completely not detected in Ghanaian adults and Italian children. Their data also provides supportive evidence that EBV infection arises later in the life of children in affluent countries than in the lives of children in poorer countries like Ghana. Thus, the age of primary EBV infection in children differs due to geographical location and co-infection

with other pathogens like *P. falciparum* and Human Immune Deficiency Virus (HIV) among others.

2.1.2.2 Sero-epidemiology

EBV maintains latency in B cells of most healthy individuals, hence it becomes imperative to determine whether EBV has a causal association with a specific disease or it is just a passer-by. Previously, heterophiles antibodies, specifically sheep agglutinin discovered by Paul and Bunnell in 1932 were used to detect the antigens of EBV. It was observed that these antibodies were highly elevated in infectious mononucleosis and not any other form of diseases, hence its use for diagnosis of EBV. These heterophile antibodies have been defined as “having the tendency to react to certain antigens which are quite different and phylogenetically unrelated to the one primarily mounting the immune response” (Paschale & Clerici, 2012). The rate at which false negative occur is greatest in children whereas false positives are only few among patients with abnormal immune response to their normal body part (Paschale & Clerici, 2012). This limitation has thus, led to the development of serological laboratory test method like Enzyme-immunosorbent Assay (EIA) to detect EBV distinct immunoglobulins as a means of diagnosis and monitoring of the infection.

Different patterns of EBV antibodies have been recognised during lytic and latent infection of health carriers as well as in viral reactivation and in numerous diseases associated with EBV. In the lytic phase for instance, regulatory proteins which belong to immediately early antigen (IEA) and early antigen are the ones being produced to ensure the synthesis of EBV DNA, virion protein structure and membrane proteins.

The lytic phase destroys the infected cells whilst releasing viral particles. EBV can likewise persist in the host's cell without completely producing virus by undergoing replication in episomes after certain selected viral genes have been expressed (Rickinson & Keiff, 2001). These genes result in perpetuation of the B cells leading to blast transformation (Tierney *et al.*, 2006; Paschale & Clerici 2012).

In the latent cycle however, EBNA1 and the three latent membrane proteins (LMPs) are being expressed. The EBNA1 comprises a complex of a minimum of six (6) proteins: (EBNA1-6). EBNA1 maintains the episomal nature of the virus DNA whereas EBNA2 is involved in immortalisation of B cell (Leight & Sugden, 2001). A study by Henle *et al.* (1978) revealed that, in Infectious mononucleosis, antibodies specific to EBNA2 were the first to arise and after attaining maximum titres, they dropped again to a very low or better still undetectable level. Anti-EBNA1 antibodies however arose after several days or weeks of decline of EBNA2 and then progressively reached titres at which they continued indeterminately. About 20%-30% of healthy individuals who have previous infection with EBV have EA (D) IgG (Bauer, 2001). In addition to high titres of VCA and IgA, EA (D), IgG are also expressed in patients with nasopharyngeal carcinoma (NPC) (Tiwawech *et al.*, 2003). Currently, antibodies against EBV immediate transactivator protein (Zta) also referred to as ZEBRA are used to diagnose individuals at a risk of having nasopharyngeal carcinoma (NPC) (Chan *et al.*, 2003; Dardari *et al.*, 2009; Njie *et al.*, 2009). EA(R) IgG has been discovered in children below the age of two (2) years, in patients suffering from eB1 and furthermore, in already infected people at a low titre (Jenson, 2004).

Parameters of EBV serology incorporate the recognition of IgM, IgG and less frequently IgA directed against EBNA_s, Viral capsid antigen (VCA) and early antigens (EA) which are partitioned into two segments: EA-D encoded by (BMRF-1) and EA-R (a human Bcl-2 homologue encoded by BHRF-1) (IARC, 1997). The IgG and (IgM) of the viral capsid antigen (VCA) together with the IgG of the EBV nuclear antigen (EBNA-1) make it easy to discriminate between early and past infection. The existence of IgM and IgG to VCA devoid of IgG to EBNA-1 is a potential indication of an early infection whereas the presence of IgG to both VCA and EBNA-1 portray past infection (Paschale & Clerici, 2012). The role of antibodies can therefore not be underestimated in terms of diagnosis of EBV infection.

2.1.3 Transmission

Epstein –Barr virus is most frequently transmitted from host to host primarily via contact with saliva (Mbulaiteye *et al.*, 2006). Infants get infected by saliva on toys, fingers, crowding and/or the act of pre-chewing of foods by their mothers (Hjalgrim *et al.*, 2007a). Saliva is predominantly the mode of transmission by which children get infected whereas intimate kissing has been proposed to be major route of transmission in adolescents (Hjalgrim *et al.*, 2007). About half of primary EBV infection during adolescent age leads to clinical infectious mononucleosis (CDC, 2006).

Breast milk has also been proposed by Ibrahim *et al.* (2015) to be another possible route of transmission among infants. Their result also showed that pregnant women who have been confirmed to have malaria during pregnancy had greater likelihood of having detectable EBV DNA than their counterpart women who lacked any sign of malaria infection in the course of pregnancy (64% vs 36%, $P= 0.01$).

Epstein- Barr virus has again been identified in cervical fluids, semen and body tissue scraped from the penile sulcus of teenage girls and young adult women and men respectively (Israel *et al.*, 1991; Elbom *et al.*, 2001) implying that sexual transmission is one of the routes of infection even though its evidence still remains elusive. According to Van *et al.* (2000), homosexuality is one of the means by which people acquire infection to EBV but this mode of transmission is limited because it is almost impossible to differentiate sexual mode of transmission from oropharyngeal contact and intimate kissing.

2.3.4 Host Range and Target Site

Even though EBV is ubiquitous in nature, it is only humans which serve as its natural host. Most of the advanced primates also possess a virus similar to EBV. The antibodies to EBV in these primates are attributed to the presence of cross-reactive antibodies against their own species-specific EBV (IARC, 2012).

Just like any other herpes virus, EBV maintains latency in lymphocytes and can stimulate proliferation of these cells. EBV is therefore said to be a B lymphotropic virus (Young & Rickinson, 2004). EBV binds to the B cell through the attachment of its outer major glycoprotein, gp350/gp220 with the cellular receptor for the C3d complement component CR2 (CD21) (Lindsey, 2007).

Not only does EBV infect B cells but also T-lymphocytes and epithelial cells resulting in distinct clinical conditions. Infection of T-lymphocytes can cause Hodgkin and non-Hodgkin lymphoma whereas infection in the epithelial cells brings about epithelial cell

tumours including nasopharyngeal and gastric carcinoma (Thompson & Kurzrock, 2004). A different CD21-independent pathway may also account for infection of other cells than the B cells (Janz *et al.*, 2000; Lindsey, 2007).

2.1.4 Pathogenesis

Epstein-Barr virus exhibits two different phases in its life cycle: the latent and lytic phase (Fig. 1). In the course of a latent infection, EBV expresses a restricted quantity of its genes that serves to stimulate the proliferation of cells, inhibition of apoptosis, blocking of viral lytic replication and also ensuring exact division of the viral episome to the daughter cells (Markus & Wolfgang, 2012). In the lytic phase however, a greater amount of the viral genes are expressed that codes for enzymes and various proteins required for nucleotide biosynthesis, RNA processing, viral DNA replication and capsid and viral envelope synthesis (Markus & Wolfgang, 2012).

Since EBV is primarily transmitted from an infected host to an uninfected host through saliva. Acute infection of EBV takes place at the oropharyngeal epithelium which happens to be the place where the lytic phase is initiated resulting in the release and amplification of viral particles (Young & Rickinson, 2004; Thorley-Lawson & Allday, 2008). Naïve B cells subsequently become infected with the virus by moving closer to the epithelial cells. The result is a manifestation of a clinically apparent or milder form of infectious mononucleosis in normal individuals. In acute EBV infection of B lymphocytes, the B cells become activated and undergo clonal expansion by using the growth transcription programme (Latency III) (Young & Rickinson, 2004; Thorley-Lawson & Allday, 2008). An EBV specific cytotoxic lymphocytes (CTL) is elicited

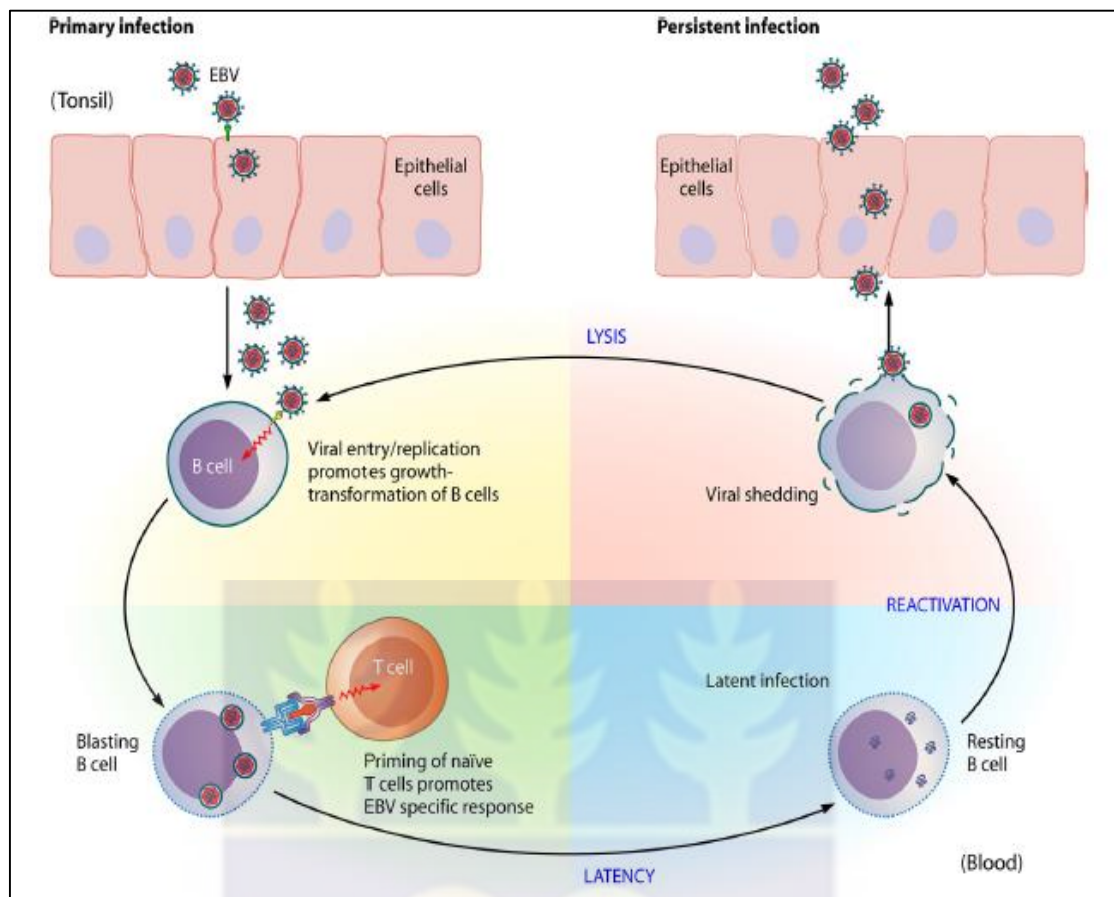


Fig. 1: Pathogenesis of EBV infection (Source: Oludare *et al.*, 2011).

which down-regulates the large number of B cells. This perhaps is the reason why EBV infected B cells decrease from a high level of about ten 10% in primary EBV infection to 1 in 10^6 cells during convalescence.

Immunoglobulins IgM, IgG and IgA are produced and these attack the viral capsid antigen of the virus during primary infection. IgA and IgG are also the two immunoglobulins produced in response to early antigen R (Jenson, 2011). In latency phase of EBV, the nuclear antigens; EBNA-3A, EBNA-3B and EBNA-3C all stimulate specific Cytotoxic T cell response against the latent proteins of the virus (Hislop *et al.*, 2007).

The virus initiates the infection of B cells by attaching its gp350/220 to the costimulatory molecule, CD21 on the lymphoid cells (Thompson & Kurzrock, 2004). The CD21 receptor molecule on the B cells become entangled and stimulates a signal which makes the naïve B lymphocytes ready for infection with EBV. After binding of the glycoprotein to CD21, a subclass of protein kinase; tyrosine kinase *lck* is immediately activated for calcium mobilisation (Raphael *et al.*, 1994; McClain *et al.*, 1995). This event is successfully followed by an increase level of mRNA production, blast transformation, homotypic cell adhesion, expression of surface CD23 and the production of interleukin (IL)-6 (Lindsey, 2007). The virus then uncoats itself and its nucleocapsid moves into the nucleus of the cell and becomes circular in nature. The circularisation of the genome and expression of W promoter result in the expression of all the EBNA proteins and the two latent membrane proteins (LMPS) being expressed (Paul, 2013). The first proteins to be recognised upon EBV infection are the EBNA-nuclear antigen leader protein (EBNA-LP) and EBNA-2 proteins. Within 1 or 2 days of infection, a shift in promoter happens which allows a C promoter (cp) to be utilized to support the underlying W promoter (Paul, 2013).

Previously, the shift in promoter was thought to occur at the same time as the switch to an expanded form of splicing that allows EBNA-3A, EBNA-3B, EBNA-3C and EBNA-1 to be expressed (Maria *et al.*, 2007). It has been established now that, expanded pattern of splicing perhaps occurs before the switch in promoter (Hughes *et al.*, 2011). This finding is parallel to a number of studies (Kume *et al.*, 1999; Wu *et al.*, 2000; Zur Hausen *et al.*, 2000) which propose that, the downstream EBNAs control the activation of C promoter. The majority of EBNA transcriptional products are involved in transcriptional control and take part in the stimulation of the viral LMP-encoding genes

expression (LMP-1 and LMP-2) and many cellular genes. The synergistic activity of these viral and cellular proteins is responsible for beginning the S-phase 1-2 days after infection (Young *et al.*, 2004). Epstein-Barr then persists in circulating subclass of memory B cells after initial infection in healthy individuals at a recurrence rate of approximately 1 in 1×10^5 to 1×10^6 cells (Thompson & Kurzrock, 2004).

The genome of the virus is mostly episomal and it exists in low levels in the nucleus of the host. Immunocompromised states permit the episomal virus to naturally replicate in the circulating B cells as seen in acute infectious mononucleosis (Thompson & Kurzrock, 2004). Healthy carriers suppress EBV latent infection through cytotoxic T lymphocytes (CTL). The absence of cytotoxic T lymphocytes may allow the development of lymphoma. Other cells such as epithelial cells, T cells and cells of the macrocytic, granulocytic and natural killer lineages are also target sites of the virus. The mechanism through which these cells become infected differs from the CD21-mediated endocytosis in B cells (Thompson & Kurzrock, 2004).

2.2 MALARIA

2.2.1 History

Malaria is an ancient disease which was originally called “mal-aria”, an Italian word which means “bad air”. It was also being called with other names such as marsh fever, ague, Roman fever, and periodic fever (Olowe *et al.*, 2015).

Back in the 1870s and 1880s, numerous scientists and researchers attempted to find the causative agent of malaria. Following Pasteur and Koch discoveries, some prominent scientist also set forth to find the bacterial cause of various diseases and malaria was no

exception (Francis, 2010). In 1879 however, the notion that malaria could be caused by a bacterium started fading out. During this time, two debatable theories were proposed regarding whether the causative agent of malaria were transmitted by either air and inhalation or by water and ingestion (Francis, 2010)

Two prominent microbiologists, Tommasi-Crudeli and Klebs claimed that, *Bacillus malariae* was the causative agent of malaria. This made Charles Louise Alphonse Laveran, an unknown French army officer to challenge their claim. He began to look out for pigments in fresh unstained blood of patients. He found that the erythrocytic stage of the organism was present in blood of malaria patients and not in healthy individuals. He realised that what he found was a protozoan malaria parasite and amidst the heated debate as to what causes malaria, his discovery led to the conclusion in 1890 that malaria was caused by a *Plasmodium* parasite (Francis, 2010). Within this period three different species of the parasite with distinct periodicity had also been discovered. These were benign tertian (*Haemoeba vivax*), malignant tertian (*Laverean malariae*) and quartan (*Haemamoeba malariae*) malaria which presently are designated as *Plasmodium vivax*, *P. falciparum* and *P. malariae*, respectively (Francis, 2010).

The search for the true vector of the malaria parasite remained an unanswerable question. In 1897, while Ronald Ross was working in India, he discovered that avian malaria parasites were transmitted by Culicine mosquitoes and thus, hypothesized that human malaria could also be transmitted by mosquitoes. It was not until 1889 that he concluded that malaria parasites were undoubtedly transmitted by *Anopheles* mosquitoes (Arora & Arora, 2008).

2.2.2 Epidemiology

Most of the malaria cases experienced in Africa are as a result of infection of *P. falciparum* and *P. vivax*. *Plasmodium falciparum* has the widest spread in Africa and it accounts for the majority of the malaria-associated death worldwide (WHO, 2015). Infection of humans is also caused by *P. ovale* and *P. malariae* and in some parts of the South- Eastern Asia, *P. knowlesi*; the parasite in monkeys (Kantele & Jokiranta, 2011). Children who are less than five (5) years of age are the most susceptible to *P. falciparum* infection resulting in high mortality and morbidity (Caline *et al.*, 2015). Pregnant women, infants, HIV patients as well as non-immune migrants, mobile populations and travellers are also considered to have a greater risk of contracting severe malaria (WHO, 2016).

2.2.2.1 Transmission

Malaria is the most predominant infectious parasitic disease of human beings which infects the erythrocyte. *Plasmodium* parasites are the causative agent of malaria and they are transmitted to humans via the bite of an infected female *Anopheles* mosquito (WHO, 2015).

According to epidemiologists, malaria can be classified as epidemic and endemic. In epidemic malaria, there is an abrupt increase in the number of cases in a defined population whereas endemic occurs when there is a definite and measurable incidence of cases over a period of years (Oaks *et al.*, 1991).

About four different malaria transmissions have been identified due the differences in transmission intensity that exist between and within malaria-endemic regions. These are

holoendemic, hyperendemic, mesoendemic and hypoendemic (Oaks *et al.*, 1991). Holoendemic region is characterised by an intense year-round malaria transmission and the immunity to malaria in this population is very high. In contrast to holoendemic, malaria transmission in hyperendemic is seasonal. This does not provide adequate immunity to all age groups. In mesoendemic however, even though there is some level of malaria transmission, epidemics can result in poor health outcome due to low level of immunity in this population. Nonetheless, hypoendemic regions have slight or certainly no immunity at all to the parasite because of its limited malaria transmission (Oaks *et al.*, 1991).

The intensity at which malaria is transmitted depends on density, longevity, biting habits and efficiency of the mosquito vector. About 25 out of the over 400 *Anopheles* mosquitoes are known to be competent vectors (Sinka *et al.*, 2012). Competent vectors, e.g. *An. gambiae* complex in Africa, have a long-life span and are also able to withstand environmental change, breed readily, occur in higher densities in tropical climates and have the affinity of biting humans. In South and Central America and Asia where transmission intensity is very low, the prevalence of *P. falciparum* and *P. vivax* are almost equal and also entomological inoculation rate is very low. In sub-Sahara Africa and parts of Oceania, transmission intensity is very high and *P. falciparum* has the highest prevalence. Entomological inoculation therefore can reach as high as 1000 per year in such areas (Gething *et al.*, 2011; Gething *et al.*, 2012).

2.2.3 Life Cycle of *Plasmodium* Parasites

The life cycle of *Plasmodium* parasites comprises of two hosts (Fig. 2): mosquito and human. Upon feeding on a blood meal of a human host, an infected female *Anopheles*

mosquito injects sporozoites into the host. These sporozoites migrate and individually infect hepatocytes within which they mature into schizonts. Upon rupture in about a week, schizonts release the merozoites within them. In *P. vivax* and *P. ovale* however, inactive stages, hypnozoites can continue to remain in the liver cells and result in recurrence by entering the bloodstream for weeks or even years. The initial replication in the liver is termed as exo-erythrocytic schizogony (CDC, 2016). The released merozoites then invade and undergo asexual multiplication in the red blood cells (erythrocytic schizogony) to form ring trophozoites which then mature into schizonts releasing more merozoites upon rupture. Some of these merozoites differentiate into blood stage gametocytes whilst others re-invade the erythrocytes and repeat the cycle.

The microgametocytes (male) and macrogametocytes (female) are taken up by a female *Anopheles* mosquito whilst sucking the blood of an infected human host. Whilst in the stomach of the mosquito, the microgametes fuse with macrogametes to form motile zygotes which become elongated and eventually form ookinetes. The ookinetes develop into oocyst after invading the wall of the midgut. The oocysts grow and release sporozoites upon rupture. The multiplication of the parasites in the mosquito is termed as sporogony. These sporozoites then find a way into the mosquito's salivary gland. Injection of the sporozoites man during a blood meal perpetuates the malaria life cycle (CDC, 2016).

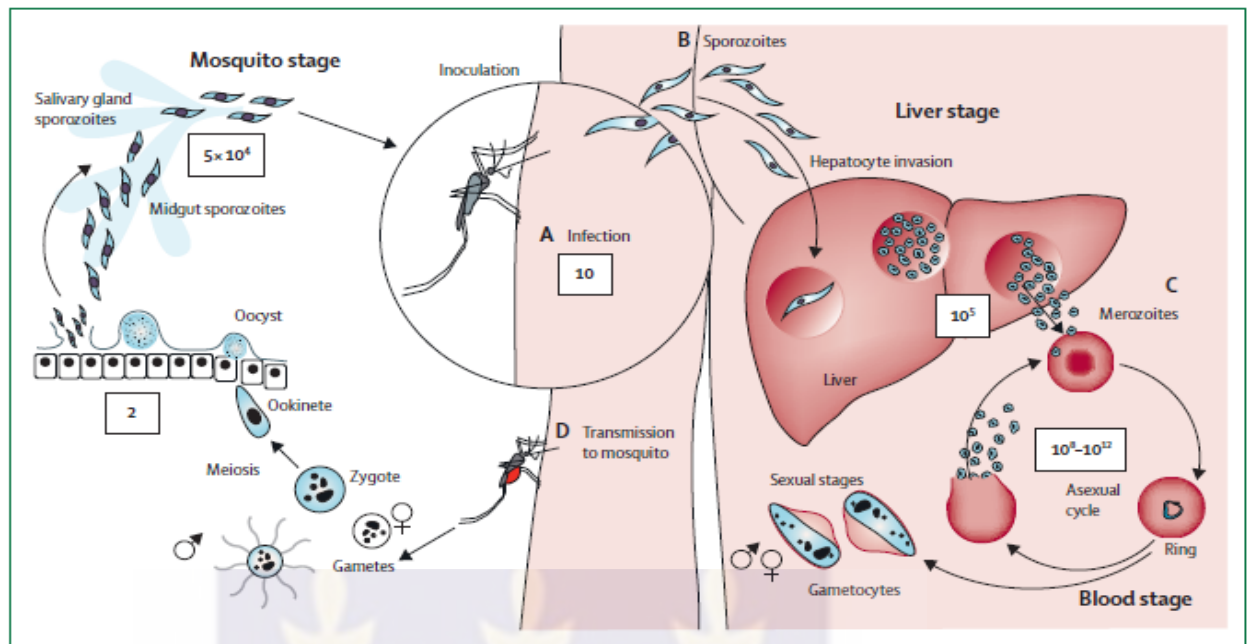


Fig. 2: Diagrammatic representation of the life cycle of *Plasmodium* parasites.
(Source: Nicholas *et al.*, 2014).

2.2.4 Symptoms

Manifestation of the symptoms of malaria is principally due to the rupture of schizonts and damage of the red blood cells by the *Plasmodium* parasite. Symptoms are felt when numbers of asexual parasites reach roughly 100 million in circulation (Nicholas *et al.*, 2014). The disease can have a gradual or a sudden and severe form with prodrome symptoms. The symptoms of malaria sometimes look like viral infection and this causes a delay in its diagnosis (Andrej *et al.*, 2003). Most patients experience fever, chills, headaches and diaphoresis. Fever is the highest symptom recorded amongst most malaria patients with diaphoresis being the lowest. Nausea, abdominal pain, dizziness, malaise are other common symptoms of the disease. Physical signs of the disease also include jaundice, pallor amongst others. Complications involve the brain, respiratory, renal and / or haematopoietic system (Andrej *et al.*, 2003).

2.2.5 Humoral Immune Response

The humoral response to malaria is one of the effective means of downregulating the level of peripheral parasitaemia in both humans and mice thereby serving as a protective immunity (Langhorne *et al.*, 2008). However, identifying which of the antibodies confer protection given the large number of the antigens present in the parasite remains elusive (Moorman, 2009). A polyclonal B cell activator, CIDR1 α antigenic structure of PfEM1 quite earlier on was identified (Chêne, 2009). Research has shown that antibodies from immune individuals recognise numerous distinct antigenic determinants in *P. falciparum* from different geographical areas (Osier *et al.*, 2008). Through the transfer of IgG from immune adults, anti-malarial immunity can be acquired which confers protection demonstrating the importance of generating malarial-specific antibody response (Dobbs & Dent, 2016).

Specific antibodies to each of the parasite stage exist. Antibodies of sporozoites specifically prevent infection by preventing them from entering the liver (Johanna *et al.*, 2014). It has been reported that, after a mosquito bite, a greater number of the parasite stay in the skin and a subset of these moves to lymph nodes (Amino *et al.*, 2006). The lymph node is a critical target site for the stimulation of protective immunity against sporozoites and also serves as the first place where sporozoite-specific CD8⁺ is recognised. Removal of the lymph node with the spleen has also been shown to totally eradicate RAS-mediated protection (Chakravarty *et al.*, 2007). B cells which happen to be target cells of EBV assume a crucial role in the production of antibodies.

Generally, when a B cell encounters an antigen, it attaches itself to the B cell receptor (BCR) causing it to be sensitized. In the context of major histocompatibility complex

(MHC) I molecule the B cell presents the antigen to T helper cell (CD4⁺) which by this process also gets activated (Boundless, 2016). This causes it to release cytokines which differentiates into T helper1 (Th1) and T helper 2 (Th2) subset development. Th1 releases cytokines like IL-10 which induces the proliferation of the B cell (Lucia & Salvatore, 2002) and consequently its differentiation that leads to the formation of short-lived plasma cells or “early memory B cells” (B cells that do not enter germinal centre) and germinal centre B cells (Zotos & Tarlinton, 2012). The germinal B cells are noted for maintain antibody titres in the circulation against circumsporozoite antigens. Memory B cells are known to keep “memory” of the parasite so as to provide immediate protective immunity in case of a secondary infection with the same *Plasmodium* parasite. According to Hviid *et al.* (2015), in stable malaria transmission areas, partial immunity is can be developed after many years of exposure and repeated infection and memory B cells may not always be a prerequisite for protection. They also identified that antibody longevity varies in an exposure- and age-dependent manner. Interestingly, the same B cells which provide immunity to malaria are the cells that serve as a target sites of EBV.

Antibody mediated immunity is thus aimed at (i) blocking parasite invasion of the RBC by agglutination of the merozoites or by blocking the interaction of ligands-receptors on the RBC surface (ii) promoting antibody-dependent cytotoxicity of infected blood cell (iRBC) mediating their target to macrophages and other phagocytic cells (iii) Blocking the activity of parasite toxins and (iv) the inhibition of the development of intraerythrocytic parasites (Langhorne *et al.*, 2008).

2.2.6 Cell-mediated Immunity

Unlike the innate immunity, cell-mediated immunity depends on previous anti-malaria immune response. The mechanisms associated with cell-mediated immunity are (i) activation of macrophage by interferon gamma (IFN- γ) obtained from natural killer cells (NK) or T helper 1 (Th1) cells for pronounced phagocytosis or killing of infected erythrocytes (Fievet *et al.*, 2001) and (ii) Priming of CD8⁺ cytotoxic and IFN- γ producing T cells that prevent the growth and development of the parasite inside the hepatocyte (Tsuji & Zavala, 2003). The severe form of malaria has been linked to the increased intensities of inflammatory cytokines such as tumour necrosis factor (TNF), interleukin-1 (IL-1) and IL-6. IFN- γ is said to have a devastating effect because of its ability to stimulate macrophages which subsequently leads to the production of TNF- α , IL-1 and IL-6 resulting in a cascade of inflammation (Leanne *et al.*, 2009). Anti-inflammatory cytokines like the tumour growth factor- β and IL-10 suppress the pathological effect of the proinflammatory cytokines. T helper cells play a very important function in the immunity of *P. falciparum* blood stages, production of IFN- γ and provision of assistance to the innate component of immunity. Cytotoxic T cells are great effectors against parasite liver stages (Sano *et al.* 2001; Overstreet *et al.*, 2008) but do not seem to participate in clearance of merozoites.

2.2.7 Immunity to Malaria in Infants

Infants seem to have partial protection from malaria infection and suffer from its consequences in the first six months of their lives. In those infants who become susceptible, infection tends to be of low parasite density and asymptomatic and gets cleared within a month (Brebini, 1990). Maternal antibodies of IgG isotypes can cross trans-placentally thereby conferring protection to the infants. *Plasmodium falciparum*

specific IgG1 and IgG3 are readily transferred from mother to child compared to IgG2 and IgG3 (Wilcox *et al.*, 2017). A study by Duah *et al.* (2010) revealed that maternal antibodies are depleted by the age of 4 months and this intend instigates the development of naturally acquired immunity or specific immunity in infants.

2.2.8 Severe Malaria

Severe malaria is said to occur when asexual stage of the *Plasmodium* parasite are detected in the blood together with a potentially fatal clinical manifestation or complication of malaria in whom other diagnoses have been ruled out (WHO, 2010).

In malaria endemic areas, severe form of the disease predominantly occurs in children and this is acquired from infancy to almost 5 years of age. Severe malaria is rare in older children and adults due to the fact that specific acquired immunity gives some sort of increasing protection. Almost 90% of the severe malaria in the world occurs in young children in sub-Saharan Africa (Black *et al.*, 2010). In patients with severe malaria, high parasitaemia and mortality increase with age. Though, severe form of malaria is more common in children it is less frequent in fatality compared with complicated cerebral malaria in children (Dondorp *et al.*, 2008).

WHO in 1990 established criteria for severe malaria so as to make it easier for future clinical and epidemiological studies (WHO, 1990). Nonetheless, in 2000, WHO made a revision to these criteria which included other clinical and laboratory findings pertaining to poor health outcome based on clinical experience in patients who have acquired partial immunity (WHO, 2000). In clinical practice however, irrespective of the WHO criteria, any individual who is suspected to have severe malaria even though not meeting

the criteria proposed by WHO is still treated as having severe malaria (Alessandro & Lorenzo, 2012)

2.3 CO-INFECTION OF EPSTEIN- BARR VIRUS AND *PLASMODIUM FALCIPARUM*

The two pathogens, EBV and *P. falciparum* are the known etiological agents of eBL. In the course of co-infection, should 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 not have happened if the pathogen was the only one (Chene *et al.*, 2011; 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 as a result of higher parasite densities compared to those with single infection and these Co-infections result mostly in reduced treatment efficacies and increased cost of treatment.

In sub-Saharan Africa, children test positive to EBV by six months age (Callan *et al.*, 1996) and EBV infected children residing in holoendemic areas in Kenya have been demonstrated by (Piriou *et al.*, 2012) to have a poorly controlled viral infection; a clue to the eBL. In another study by Chattopadhyay *et al.* (2013), exposure to holoendemic malaria has been associated with alteration in the differentiation of Epstein-Barr virus – Specific CD8⁺ T cell. Most studies (Moorman *et al.*, 2005; Rasti *et al.*, 2005, Piriou *et al.*, 2005) that investigated co-infection of EBV and malaria established the fact that co-infection with these two pathogens is responsible for eBL.

The association between co-infection of EBV and *P. falciparum* with severe malaria in children on the other hand has not gained much popularity compared to its association with eBL. In spite of this, in a study by Caline *et al* (2015), mouse models of EBV (murine gamma herpes virus 68 (MHV68) and malaria (*P. yoelii* XNL)] were used to elucidate that acute gamma herpes virus infection can dysregulate the production of antibodies that regulate *Plasmodium* parasites and then cause a non-severe *P. yoelii* XNL infection to become severe, eventually leading to death. This points out that asymptomatic primary acute EBV infection and recurrent malaria infections can lead to severe malaria in children (Caline *et al.*, 2015).

A study by Wedderburn (1988) also showed that marmoset *Callithrix jacchus* infected with *P. brasilianum* and EBV had higher parasitaemia than did the animals infected with only *P. brasilianum*, the outcome of which was glomerulonephritis. In *P. falciparum* endemic regions, data from mathematical models suggests that, it is only after 1-2 infections with *P. falciparum* that acquisition of immunity to severe non-cerebral malaria in children can be attained (Langhorne *et al.*, 2008).

2.3.1 Gamma Herpes Virus and Malaria Immunity

A breakthrough study which involved the use of a mouse model co-infected with murine herpes virus (MHV-68) and *P. chabaudi* or *P. 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 (Vigin & Speck, 1999; 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 transforming into a lethal one.

The consequences of acute MHV68 on the pathogenesis of malaria seemed to be evident from the changes in antimalarial adaptive immune response which is generally driven by innate immunity. Gamma herpes virus ability to regulate the humoral immunity of malaria may have interesting inference on the immunobiology of malaria during co-infection (Fig. 3) (Matar *et al*, 2015). The use of a mouse model, C57BL/6 infected with 1000PFU of MHV-68 and 10^5 parasitized erythrocytes of *P. yoelii* XNL or *P. chabaudi* AS has proven that, infection with either *Plasmodium* was not severe but amid co-infection with MHV68 mouse, *P. yoelii* caused 100% deaths (Matar *et al.*, 2015).

Cytokines and chemokines play a fundamental part in humoral immunity to the pathogen. Gamma herpes virus just like *Plasmodium* also induces type 1 IFN production from antigen presenting cells (APC) through ligation of Toll-like Receptor (TLR) 9 by DNA/protein complexes confined in haemozoin crystals (Parroche *et al.*, 2007; Wu *et al.*, 2010). Type I IFN has been demonstrated to stimulate haematopoietic stem cell exhaustion in *Plasmodium* infection (Essers *et al.*, 2009; Sato *et al.*, 2009) and in a context with viral infection, type 1 has been proven to have inhibitory properties on haematopoiesis during infection with lymphocytic choriomeningitis virus (LCMV) (McNab *et al.*, 2015). This proposes a possible role for chronic type 1 IFN signaling in aggravating severe malarial anaemia, a condition where erythropoeitic and haematopoietic suppression is a hallmark (Lamikanra, 2007). In a study using *P. berghei* Anka model of malaria, type 1 IFN has been shown to suppress its CD4⁺ T cell response (Haque, 2004). The phenomenon by which this occurs still remains vague.

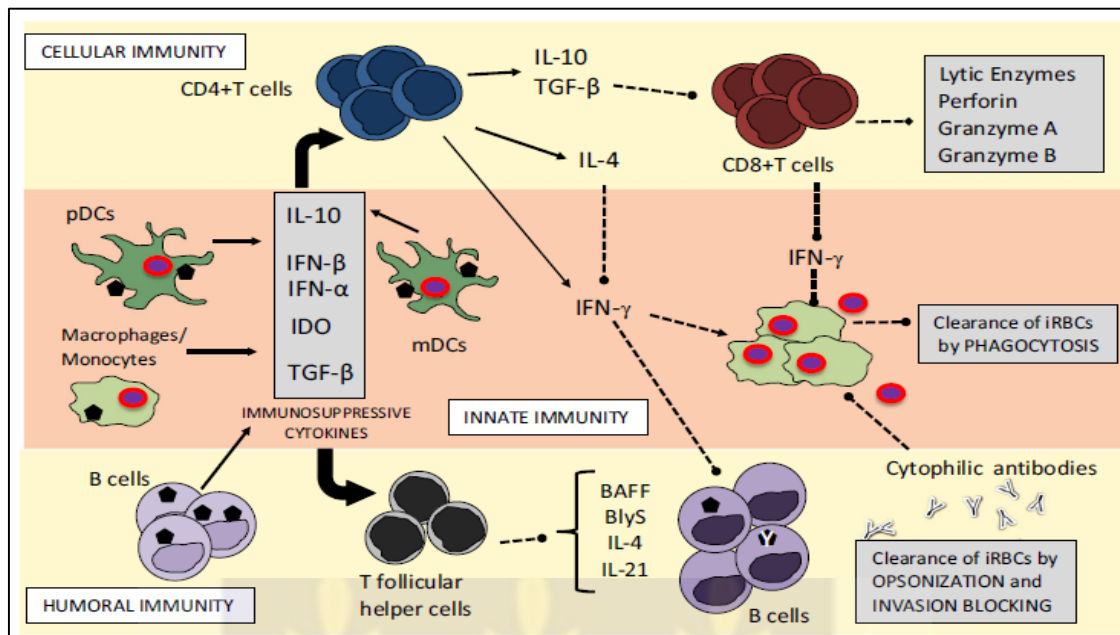


Fig. 3: A model of how EBV interferes with malaria immunity. (Source: Matar *et al.*, 2015).

Parasitized red blood cells are indicated by the purple circles with red outline. Points of enhancement are represented by solid lines and suppression by broken lines. EBV is indicated by black pentagons

However, a school of thought has proposed that, it is likely due to an effect on antigen presentation cells (APC) which activate T-helper cells. MHV68 –infected dendritic cells have been found not to be able to up-regulate activation markers and provide efficient antigen presentations compared to those unexposed to MHV68 (Smith *et al.*, 2007).

Similarly, infection of monocytes by EBV is known to prevent their maturation into dendritic cells and eventually promoting apoptosis (Li *et al.*, 2002). IL-10 has been found to circulate in the plasma of patients with acute malaria (do Rosário *et al.*, 2012). Infection with a gamma herpes virus has also been known to up-regulate levels of IL-10. The MHV68 achieve this through the M2 gene product (Moorman *et al.*, 2007; Moorman *et al.*, 2009). EBV codes for a viral IL-10 homolog (Slobedman *et al.*, 2009) and EBV -

induced IL-10 production is proposed to increase the proliferation of B-cell thereby leading to the expansion of the latency reservoir. The cytokine, IL-10 has the ability to immunosuppress TH1 responses (Saraiva & Garra, 2010) and IL-10 can impair the immunity to *Plasmodium falciparum* infection. Nonetheless, IL-10 has a vital role in the protection against extensive inflammatory response which leads to the pathogenesis of malaria (Freitas *et al.*, 2012).

IL-10 has been associated with the stimulation of the enzyme, 2, 3 dioxygenase (IDO). This enzyme is responsible for catalyzing the catabolism of tryptophan (TRP) via the kynurenine pathway (Munn & Mellor, 2013). IDO also negatively modulates Th1 CD4⁺ / CD8⁺T cells whilst stimulating the CD4⁺ FOXP3 T regulatory subset. Nonetheless, there is no corroboration that either of the T cells or T regulatory cells is transformed during plasmodium and murine herpes virus co-infection in the mouse. This implies that, the reason for the antagonistic effect of MHV68 on antibody-mediated immunity to malaria is not due to IDO (Matar *et al.*, 2011).



CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

A cross sectional study was conducted among children with malaria. These children were further grouped into those who had severe malaria and those who did not have (non-severe malaria). Those with non-severe malaria were used as a comparison group to detect whether any association existed between severe malaria and Co-infection with EBV.

3.2 STUDY SITES

The study was carried out at Divine Love hospital (DLH) at Bibiani (Western Region) and Nkenkensu Government hospital (NKG) at Nkenkensu (Ashanti Region). Bibiani is a town and capital of Bbiani/Anhwiaso/Bekwai district. The district is positioned in the north-eastern part of the Western region between latitude 6° N, 30° N and longitude 2° W, 30° W (Bibiani-Anhwiaso-Bekwai District Assembly, 2012). Divine Love hospital is a general private hospital and is amongst the only four hospitals in the district (Bibiani-Anhwiaso-Bekwai District Assembly, 2012). The hospital provides healthcare services to neighbouring towns like Abesinsuom, Gyedi, Degede, Atakrom and others. Although Bibiani is a mining centre, most of its indigenes are in the agricultural sector. Malaria is still one of the main health concerns in this district (Bibiani-Anhwiaso-Bekwai District Assembly, 2012).

Nkenkensu is one of the sub-districts in Akomadan, the capital town of the Offinso-North District. The district lies in the northern part of the Ashanti region with a population of about 56,881 (28,581 females and 28,300 males) [Offinso -North District

Assembly, 2015]. The population is concentrated in the principal towns of Akomadan, Nkenkensu and Afrancho which are the urban towns. The predominant activity of the people is agriculture (Offinso -North District Assembly, 2015). Nkenkensu Government Hospital is the district hospital for Offinso-North. It was established in 1966 and it was initially a health centre. As at June, 2011, 22, 624 outpatient cases, 1277 admissions and 34.3% of the total deaths in the district were attributed to malaria (Offinso -North District Assembly, 2012).

3.3 STUDY POPULATION AND SAMPLING METHOD

The study population comprised of children below (5) years of age who had been clinically diagnosed and confirmed to have malaria at the Divine Love and Nkenkensu Government hospitals. Convenient sampling was used to enrol the children from the two study sites after informed consent had been obtained from their parents/guardian.

3.3.1 Inclusion Criteria

Inclusion criteria were:

- i. Children under 5 years
- ii. Children clinically diagnosed to have malaria and confirmed to have malaria by microscopy
- i. Children both sickle cell and HIV negative

3.3.2 Exclusion Criteria

The exclusion criteria were:

- i. Children above 5 years of age

- ii. Children who had apparent infection besides malaria or pyrexia of unknown origin
- iii. Both HIV and sickle cell positive children

3.5 SAMPLE SIZE CALCULATION

The sample size was determined using the statistical formula as described by (Daniel, 1999);

$$n = \frac{[Z]^2 P(1 - P)}{E^2}$$

where Z =1.96 is the standard score for the confidence interval of 95%

P, the sample proportion of EBV seroprevalence in Ghanaian children. Since there is no specific EBV seroprevalence among Ghanaian children, an assumed prevalence of 50% was used.

Using a 9% allowable error (E),

$$n = \frac{[1.96]^2 (0.5)(1 - 0.5)}{0.09^2}$$

=118 children

3.6 ENROLMENT AND GROUPING OF THE CHILDREN

Any child brought to the laboratories in the two hospitals upon a clinician's request for blood film and met the inclusion criteria and whose parents/guardian consented and signed (Appendix I) for the child to participate in the study was enrolled. An identification number was allotted to each of the children and data on demographics was obtained by a questionnaire (Appendix II) administered to the parents/guardians. Clinical and laboratory data was also obtained from the folders of the children. All children were recruited within a period of 3 months; April to June, 2017.

3.7 HAEMATOLOGICAL AND PARASITOLOGICAL ANALYSIS

3.7.1 Blood Collection and Processing

A minimum of 500 µl of venous blood was collected into EDTA tubes from each child. The blood was subjected to thin film, HIV and sickling test after which plasma was separated from red cells using a Hettich Universal 16A centrifuge at 1500 rpm for 5 minutes. The plasma was then transferred into labelled Eppendorf tubes and stored at -20°C until further analysis.

3.7.2 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 and HIV status were also determined for the participants. Each laboratory's standard operating procedures were followed.

3.7.2.1 Sickling test

Sickling test was carried out by the sodium metabisulphite technique. A drop of the patient's venous blood was put on a slide and an equal volume of 2% sodium metabisulphite was added, mixed and then covered with a cover glass and left for 10-15 minutes. The 10× objective lens was first used for focusing and then 40× objective lens was used for examination of the slide under the microscope.

3.7.2.2 Identification of *P. falciparum* by thin film

Thin film was used in differentiating *P. falciparum* from other *Plasmodium* parasites. A drop of blood was placed at the centre of a labelled grease - free slide and then was spread to have a smooth end. The slides were made to air-dry and then fixed with absolute methanol. Afterwards, the slides were first stained with Field stain B and then an equal volume of field stain A was added to the slides. The slides were left for 60 seconds to stain. Stains were washed off with clean water and made to air-dry. A drop of oil immersion was put on each slide and then observed under the Olympus light microscope using the 40× objective lens for focusing and 100× objective lens for examining.

3.7.2.3 Estimation of *P. falciparum* density by thick film

Thick film was used in the estimation of *P. falciparum* density in the blood. A small drop of blood was placed at the centre of the slides and then spread to make a thick smear. The smears were air-dried after which each of them was dipped into Field stain A for 5 seconds. They were then gently washed with clean water and then dipped into field stain B for 3 seconds. Excess stain was drained off and the slides again washed with clean water. The backs of the slides were wiped off and then made to air-dry. A drop of immersion oil was then applied onto the slides. The slides were first observed using 40× objective lens and then finally the 100× objective lens. Parasites were counted against a pre-determined WBC of $200 \times 10^9/l$ described in Monica (1998) using the formula;

$$\text{Parasites}/\mu\text{l} = \frac{\text{Parasite count} \times 8000}{\text{Number of WBCs counted}}$$

3.7.2.4 Detection of HIV antibodies

The First Response HIV 1-2.O rapid test (Premier Medical Corporation Ltd., India), was used to detect the HIV status of each participants according to the manufacturer's protocol. One or 2 drops of whole blood were added to the sample well using the sample pipette. A drop of the assay buffer was also added to the sample well. Results were interpreted after 15 minutes.

3.7.3 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). The kit according to the manufacturer has a diagnostic specificity of 100% and a sensitivity of 98%.

3.7.3.1 Assay procedure

Prior to assaying, all samples and reagents were brought to room temperature. A 90 microtiter well plate was used and one well each was allocated for the substrate blank (A1), negative control (B1) and positive control (E1). Well C1 and D1 were allocated for cut-off controls.

Each participant's sample was diluted by adding 1 ml of sample diluent to 10 µl of serum specimen in Eppendorf tubes. The diluted samples were thoroughly mixed and incubated for 15 minutes at room temperature (25°C). For each run, 100 µl each of diluted samples, negative and positive controls was dispensed into the designated wells with new disposable pipette tips. The wells were covered with a foil and then incubated

for 1 hour at 37°C. The contents of the plates were discarded and each well was then rinsed 5 times with 300 µl of wash solutions.

Residual droplets were removed from the wells onto absorbent paper and 100 µl of the enzyme conjugate dispensed into each well except the blank well. The plate was then incubated for 30 mins at room temperature. The wells were again rinsed 5 times with a diluted wash solution (300 µl per well). Then a 100 µl of the substrate solution was added into all wells followed by incubation in the dark for exactly 15 minutes at room temperature.

3.7.3.2 Measurement

The enzymatic reaction was stopped by adding 100 µl of the stop solutions into each well. The absorbance at 450 nm was read within 30 minutes using Multiskan MS microtiter plate reader (Thermo Labsystems Inc., US) after adding stop solution. The mean absorbance value for some of the samples which were duplicated was calculated.

3.7.3.3 Results

3.7.3.3.1 Validation

The test run was considered valid after meeting the validation criteria set by the manufacturer of the assay kit.

3.7.3.3.1 Calculation

The mean absorbance value of the two cut-off controls was calculated and used to calculate the optical density (OD) of each sample.

3.7.3.3.1 Interpretation

An OD of greater than or equal to 0.416 was interpreted as positive whereas an OD less than 0.34 was interpreted as negative. Optical density that was less than 0.416 or equal to 0.34 was interpreted as grey zone.

3.8 STATISTICAL ANALYSIS

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

Differences between severe and non-severe malaria groups in terms of clinical manifestations and haematological profile were tested. EBV infection between children with and without severe malaria was also compared. Analyses were carried out using IBM® SPSS® Statistics version 24 for Windows and GraphPad Prism version 7.00 for Windows. 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.

3.9 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. Approval

was also sought from the management of the Divine Love and Nkenkensu Government hospitals. Parents and/or guardians of the children were provided with written consent forms to complete.



CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC DATA OF THE CHILDREN

A total of 80 children under 5 years were enrolled into this study. The minimum and maximum ages were 1 month and 48 months respectively, with a mean of 26.48 ± 14.67 months. Of the 80 children, 50 (62.5%) were recruited from Divine Love Hospital whereas the remaining 30 (37.5%) were from Nkenkensu Government Hospital. In all, there were 49 (61.25%) children classified as having severe malaria and 31 (38.75%) children with non-severe malaria (Table 1). No statistically significant differences were observed between the two groups in relation to age groups ($p = 0.8896$) and gender ($p = 0.1650$).

There was no significant difference ($p = 0.0819$) between the use of insecticide- treated net (ITN) among the two groups, severe and non-severe malaria



Table 1: Demographic information of the two groups of children

CATEGORY	VARIABLE	MALARIA GROUP		P- VALUE
		NON-SEVERE	SEVERE	
		(N = 31)	(N = 49)	
AGE in months (Mean ± SEM)		27.61 ± 2.652	25.76 ± 2.101	0.5842
AGE GROUPS (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 ITN	Yes	21 (40.45)	31 (59.6)	0.0819
	No	10 (35.7)	18 (64.3)	

*The figures in parenthesis represent percentage (%)

4.2 CLINICAL HISTORY

Table 2 shows the clinical history of the children in the two malaria groups. Both groups had similar clinical histories: diarrhoea, vomiting, pallor, malaise, respiratory distress, convulsion and fever. However, there were differences in some of the histories recorded. Fever was present in all the children in the severe malaria group whilst malaise was recorded in only one child in the non-severe group.

Statistically significant differences were observed between the two groups for some of the clinical histories. Core body temperature, duration of illness, fever and malaise, for example, showed statistically significant differences (all p s < 0.05) between the two groups.

4.3 HAEMATOLOGICAL ANALYSES

Table 3 shows the haematological analyses of the children in the two malaria groups. Statistically significant differences were observed between the two groups for some of the haematological parameters. WBC and parasite density (Fig. 4), for example, showed statistically significant differences (both p s < 0.05), compared to both RBC and Hb concentrations (both p s > 0.05) between the two groups.

Table 2: Clinical histories of the two groups of children

CATEGORY	VARIABLE	MALARIA GROUP		P-VALUE
		NON-SEVERE (N = 31)	SEVERE (N = 49)	
CORE BODY				
TEMPERATURE (°C)		37.55 ± 0.1503 ^a	38.4 ± 0.09184 ^a	0.0001*
DURATION OF ILLNESS (Days)		1.613 ± 0.2004 ^a	2.286 ± 0.2278 ^a	0.0436*
FEVER	Yes	15 (48.39)	49(100)	<0.0001*
	No	16(51.61)	0(0)	
DIARRHOEA	Yes	8(25.81)	19(38.78)	0.3320
	No	23(74.19)	30(61.22)	
VOMITING	Yes	15(48.39)	32(65.31)	0.1650
	No	16(51.61)	17(34.69)	
PALLOR	Yes	5(16.13)	18	0.0748
	No	26(83.87)	31	
MALAISE	Yes	1(3.23)	22(44.90)	<0.0001*
	No	30(96.77)	27(55.10)	
RESPIRATORY DISTRESS	Yes	3	7	0.7328
	No	28	42	
CONVULSION	Yes	0(0)	2 (4.08)	0.5193
	No	31(100)	47(2.35)	

*Significant at <0.05; ^aMean ± SEM

Figures in parenthesis represent percentage (%) with

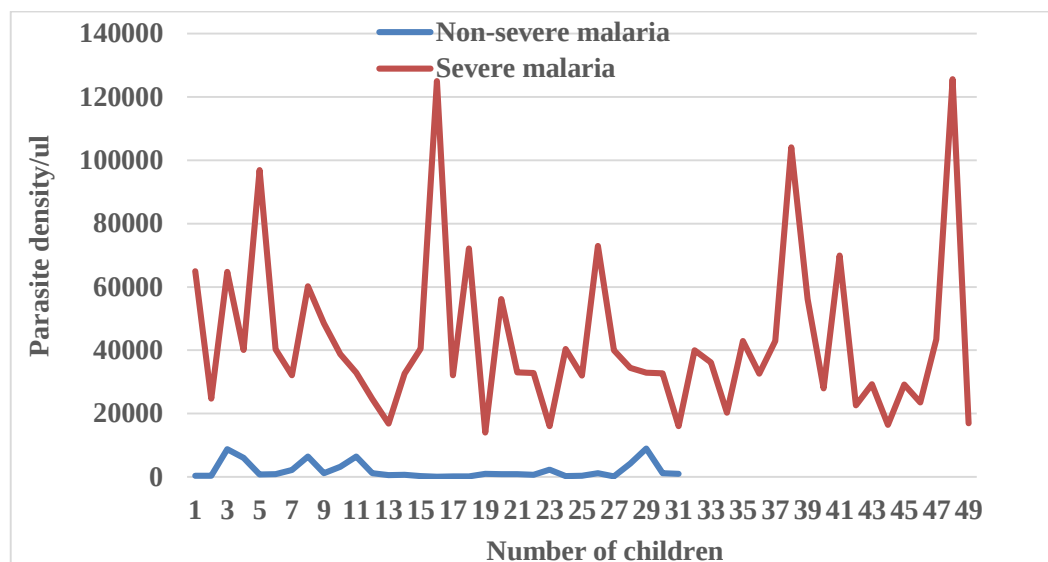


Fig. 4: Parasite density levels between the two groups of children.

Table 3: Haematological parameters of the two groups of children n

CATEGORY	MALARIA GROUP		P-VALUE
	NON-SEVERE ^a	SEVERE ^a	
	(N = 31)	(N = 49)	
HB (g/dl)	8.332 ± 0.241	8.218 ± 0.2382	0.7497
WBC (10 ⁹ /L)	7.919 ± 1.299	12.24 ± 1.066	0.0126*
RBC (10 ¹² /L)	4.529 ± 0.3982	4.005 ± 0.1178	0.1374
Parasite density (/μl)	2009 ± 462.6	43293 ± 3718	<0.0001*

*Significant at <0.05; ^aMean ± SEM

4.4 DETECTION OF EPSTEIN-BARR NUCLEAR ANTIGEN-1 (EBNA-1) IgG

Figure. 5 shows the distribution of EBV among the two malaria groups. The overall seroprevalence of EBV in this study was 26.25%. 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. The seroprevalence of EBV among children within the age range of 8-23 months was 25% whilst the prevalence of EBV in children within the age range of 24-48 months was 28.57%. No EBNA-1 IgG was found in children between 0-7 months.

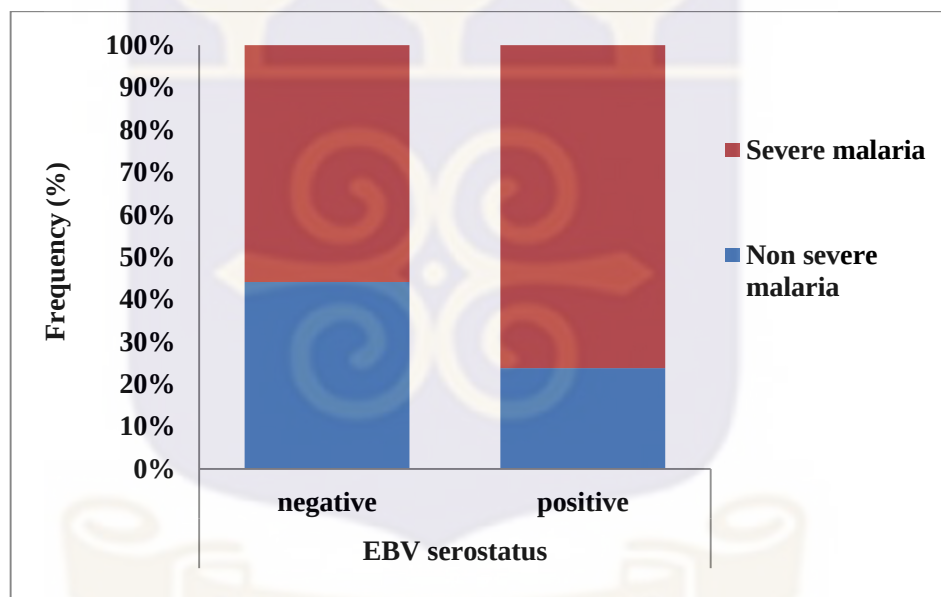


Fig. 5: Distribution of EBV among the two malaria groups

Out of the total 33 females, 8 were EBV seropositive and 25 were EBV seronegative. Of the 47 males, 34 were seronegative to EBV whereas the remaining 13 were seropositive. There was no significant association between gender and EBV serostatus ($p = 0.73$).

4.5 CORRELATION BETWEEN EBV AND BOTH CLINICAL HISTORIES AND HAEMATOLOGICAL PROFILES OF THE TWO GROUPS OF CHILDREN.

Tables 4 and 5 show the correlations between EBV and both clinical histories and haematological profiles of the children, respectively. No significant correlations (all $p > 0.05$) were observed in all cases.

Table 4: Correlation between EBV and haematological profiles of the children

	Parasite density	Hb	RBC	WBC
r	0.2017	0.1463	-0.1565	-0.0576
95% confidence interval	-0.01886 to 0.4035	-0.07586 to 0.3546	-0.3637 to 0.06552	-0.2739 to 0.1642
p value	0.0728	0.1954	0.1658	0.6118

Table 5: Correlation between EBV and clinical histories of the children

	Duration of illness (days)	Fever	Diarrhoea	Vomiting	Pallor	Malaise	Respirat- ory distress	Convulsion
r	0.048	- 0.156	0.175	0.187	-0.065	0.186	-0.0514	0.086
95% confidence interval	-0.173 to 0.2654	- 0.363 to 0.065	-0.04652 to 0.3801	-0.0337 to 0.3909	-0.2808 to 0.1568	-0.0352 to 0.3897	-0.268 to 0.1703	-0.1359 to 0.3004
p value	0.669	0.166 3	0.1205	0.0961	0.566	0.0986	0.651	0.4458

4.6 ASSOCIATION BETWEEN THE SEVERE MALARIA GROUP AND EBV

Though there was no significant difference between EBV serostatus and malaria ($p = 0.123$), among the two malaria groups, EBV coinfection was likely to lead to severe *falciparum* malaria development (Risk ratio 2.024, 95% CI 0.8249 to 4.9686, $p = 0.1236$).



CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

There is robust evidence that, EBV is essential but it cannot on its own suffice to cause endemic Burkitt's lymphoma in children. It needs a cofactor, which is *P. falciparum* which destroys immunity to EBV thereby resulting in a large number of B cells being infected with EBV (Moormann *et al.*, 2011), a hallmark of Burkitt's lymphoma. The cofactor, *P. falciparum* has by far been ascribed 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 5 years.

In the current study, there were more males than females but the difference in gender was not statistically significant ($p=0.89$). This implied that gender might not be a risk factor for malaria. A study in Uganda by Danielle & Glenda (2016) also found a similar result. There was also no significant difference ($p=0.58$) between mean ages of those grouped as having severe malaria (25.76 ± 2.101) and those without severe malaria (27.61 ± 2.652). The absence of any statistical difference in the ages makes it easier to make comparison between severe and non-severe malaria group without any bias. There was also no significant difference ($p=0.89$) between the age groups of those with severe and non-severe malaria.

There was no significant association ($P = 0.082$) between the use of ITNs for the two malaria groups. This finding is parallel with the findings of Iwuafor *et al.* (2016) but contradicts the findings of Lengler (2004) who reported that, the use of mosquito net can help curb malaria morbidity and mortality in children. In Gambia for instance, a study by Choi (1995) also showed that the use of ITNs reduced about 50% of the incidence of severe malaria leading to death. A study in Benin by Damien (2001) and another study in Tanzania by (West *et al.*, 2013) did not also observe any association between ITNs and *falciparum* malaria infection. The absence of any statistically difference between use of ITNs by severe and non-severe malaria groups probably means that, the two groups were equally exposed to the bite of mosquitoes.

There were significant differences in the clinical histories for fever, core body temperature malaise and pallor between participants with severe malaria and those with non-severe malaria. These were probably due to the fact that participants with severe malaria had significantly higher parasite densities ($p < 0.0001$) than those with non-severe malaria. This finding is in agreement with the findings of Bouvier *et al.* (1997) who also found out that high parasite density is a likely indicator of an incidence of fever. Owing to this, it can be said that, the parasite density might also be the reason for a significant difference in the clinical histories between the two groups. Also, a significant difference ($p = 0.04$) between duration of illness prior to attending hospital amongst the children with severe malaria and non-severe malaria was observed. This finding agrees with findings of Hassan *et al.* (2008) who also reported that patients, with prolonged duration of illness had a higher probability of having higher levels of parasitaemia.

The significant differences ($p=0.013$) in white blood cell counts observed between severe and non-severe malaria groups was probably also due to differences in the level of parasitaemia between the two malaria groups. This finding agrees with the report of Mckenzie *et al.* (2005), Abro *et al.* (2008) and Simji *et al.* (2017) who also found an association between WBC and parasite density.

In this study, EBNA-1 IgG was detected in the plasma of both children with severe or non-severe malaria. There seems to be no general prevalence of EBV in children because age-specific EBV antibodies in children vary from 20% to nearly 100% and this is dependent on race/ethnicity and geographical location (Balfour *et al.*, 2013; Lawrence *et al.*, 2014).

Thus, overall prevalence of 26.25% in this study was lower compared to even the prevalence of 35% in infants who were 6 months old in a holoendemic area in Kenya (Piriou, 2012). A study by Lawrence *et al.* (2014) also found EBV prevalence of 25%-52% among non-hispanic black children between the ages of 1-5 years. In United States, 31% of children were found to be seropositive to EBV (Condon *et al.*, 2014). With regards to the hypothesis by de Thé *et al.* (1978) that almost all children would have seroconverted by the age of 2 years in developing country; it implies that, the overall seroprevalence in this study was lower. However, the low seroprevalence of EBV in this study could be attributed to the fact that only EBNA-1 IgG was used as an indicator of EBV infection as against other studies that used a variety of EBV antibodies to estimate the prevalence of infection.

The age-specific prevalence found in this study is lower compared to the age-specific prevalence found among children in Thailand (Rabporn *et al.*, 2015). The lower age-specific prevalence is probably due to the fact that anti-VCA IgG was used in the detection of EBV in Thailand children whereas EBNA-1 IgG was used in this study. Anti-VCA IgG is a marker of recent infection whilst Anti-EBNA-1 IgG is a marker of past infection (Paschale & Clerici, 2012). It is likely that most children under 5 years are having recent infection rather than past infection; hence the prevalence of Anti-VCA in children in Thailand was greater than the seroprevalence of EBNA-1 IgG in this study. Besides, the prevalence in this study was specific to children presented with only malaria. The absence of EBV infection in the 0-7 months age group probably means none of the children within this age range was infected and therefore did not have maternal antibodies to EBNA-1 in their blood circulation.

The study did not find any significant association between EBV serostatus and the degree of malaria ($p = 0.123$). This implies that Co-infection of EBV and *P. falciparum* in children with malaria was not linked to severity of malaria in this study. The study did not also find any significant association ($p = 0.73$) between gender and EBV serostatus even though the number of males who seroconverted were 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.*, 1996; Sdorchuk *et al.*, 2003; Adjei *et al.*, 2008; Balfour *et al.*, 2013). Male seropositive rate being higher than female seropositive rate can also be explained by the fact that, there were more males than females in the study.

The lack of significant association ($p=0.123$) between co-infection of EBV and *P. falciparum* in this study might be because the EBNA-1 is in line with the findings of

Matar *et al.* (2015) who by using mouse model elucidated the impact of co-infection of EBV and *P. falciparum* on the severity malaria, also found that latent EBV infection was not associated with severe malaria.

One of the limitations of this study was the small sample size used. This was probably one of the reasons for the study's lack of power to detect many significant associations from this data. Secondly, the use of EBNA-1 IgG alone was not enough for EBV detection in children. Thus, VCA IgG and IgM should have been tested alongside EBNA-1 IgG.

5.2 CONCLUSIONS

The study determined that even though children with severe malaria had higher seroprevalence of EBNA-1 IgG, this was not responsible for malaria severity since children with non- severe malaria also had EBNA-1 IgG present in their blood plasma.

The study also determined that EBV was not responsible for the differences in fever, malaise and WBC observed between children with severe and non-severe malaria since there was no correlation between EBV, clinical history and haematological parameters.

Children with EBV were however, more likely to progress to severe malaria.

It is recommended that:

1. Further research is needed to investigate the correlation between EBV and severe malaria in children under 5 years using combination of antibodies to latent and lytic antigens.

2. There should be more longitudinal research in humans to study the impact of EBV on severity of malaria.
3. A larger sample size can also be used to determine the correlation between EBV infection and severe malaria in future studies.



REFERENCES

- Abro AH, Abdalla MU, Nadeem JY, Ahmed AS, Dujana A & Ahmed AS (2008) Malaria and haematological changes. *Pakistani Journal of Medical Sciences*, 24: 287-291.
- Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, *et al.* (2008) Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. *BMC Infectious Disease* 8: 111.
- Alessandro B & Lorenzo Z (2012) Clinical aspects of uncomplicated and severe malaria. *Journal of Haematology in Infectious Diseases*. 4(1): e2012026
- Amino R, Thiberge S, Martin B, Celli S, Shorte S, Frischknecht F & Menard R (2006). Quantitative imaging of Plasmodium transmission from mosquito to mammal. *Nature Medicine*, 12: 220–224.
- Andrej T, Matjaz J, Igor M & Rajesh MP (2003). Clinical review: Severe malaria *Critical Care*. 7: 315.
- Anumudu CI, Okafor CM.F, Ngumohaike V, Afolabi KA, Nwuba RI & Nwagwu M (2004). Clinical manifestation and immunological response in severe paediatric malaria in adeoyo hospital, Ibadan. *African Journal of Medicine and Medical Sciences*, 33(1):57-63.
- Arnaud C, Daria D, Jackson O, Anders B, Mbidde ER, Fred K *et al* (2009). Endemic Burkitt's lymphoma as a polymicrobial disease: New insights on the interaction between *Plasmodium falciparum* and Epstein–Barr virus. *Seminars in Cancer Biolog*, 19(6):411-420.
- Arora DR and Arora B (2008). Medical Parasitology. 2nd edition. CBS Publishers & Distributors. pp.67-76.
- 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.
- Bauer G. (2001). Simplicity through complexity: immunoblot with recombinant antigens as the new gold standard in Epstein-Barr virus serology. *Clinical Laboratory*. 47:223–230.

- Bennett NJ & Domachowske J (2010). Pediatric mononucleosis and Epstein-Barr virus infection. eMedicine from WebMD. Available from: <http://emedicine.medscape.com/article/963894-overview>.
- Bibiani-Anhwiaso-Bekwai (2012). The Composite Budget of the Bibiani-Anhwiaso-Bekwai district assembly for the 2012 fiscal year. MTEF Budget document.
- Biggar RJ, Henle W, Fleisher G, Böcker J, Lennette ET & Henle G (1978). Primary Epstein-Barr virus infections in African infants. Decline of maternal antibodies and time of infection. *International Journal of Cancer*, **22**(3):239–43.
- Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG *et al.* (2010). Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*, 375:1969–1987.
- Boundless (2016)."Humoral Immune Response." Boundless Biology Boundless. Retrieved 17 Jun. 2017 from <https://www.boundless.com/biology/textbooks/boundless-biology-textbook/the-immune-system-42/adaptive-immune-response-234/humoral-immune-response-875-12125/>.
- Bouvier P, Rougemont A, Breslow N, Doumbo O, Delley V, Dicko A *et al.* (1997). Seasonality and malaria in a west African village: does high parasite density predict fever incidence. *American Journal of epidemiology*, 145(9):850-7.
- Brebin B (1990). An analysis of malaria parasite rates in infants: 40 years after McDonald. *Tropical Disease Bulletin*, 87:R1-R21.
- Buisson M, Morand P, Genoulaz O, Bourgeat M J, Micoud M & Seigneurin JM. (1994). Changes in the dominant Epstein-Barr virus type during human immunodeficiency virus infection. *Journal of General Virology*, **75** (Pt. 2): 431–437
- Buttler NS, Harris TH & Blader IJ (2013). Regulation of immunopathogenesis during *Plasmodium* and *Toxoplasma* infections: more parallels than distinctions? *Trends in Parasitology*. 29: 593-602.
- Byakika-Kibwika P, Ndeezi G & Kamya M (2009). Health care related factors associated with severe malaria in children in Kampala, Uganda. *African Health Sciences*, 9(3):206–210.
- Caline GM, Neil RA, Brigid MOF, Nathan TJ, Lalita P, Christian RE, Samuel HS & Tracey JL (2015). Gamma herpes virus co-infection with malaria suppresses anti-parasitic humoral immunity. *Plos Pathogens*, 11(5): e1004858.

- Callan MF, Steven N, Krausa P, Wilson JD, Moss PA, Gillespie GM *et al.* (1996). Large clonal expansions of CD8⁺ T cells in acute infectious mononucleosis. *Nature Medicine*, 2: 906–911. PMID: 8705861
- CDC (2006). Epstein-Barr Virus and infectious mononucleosis. Atlanta, GA: Centers for Disease Control and Prevention. Available at: <http://www.cdc.gov/ncidod/diseases/ebv.htm>
- CDC (2016). Malaria: Biology: Centers for Disease Control and Prevention. Available at: <http://www.cdc.gov/ncidod/diseases/ebv.htm>
- Centre of Disease Control and Prevention (2015). Malaria. www.cdc.gov/malaria/about/disease.html. accessed on 6th June 2016.
- Chakravarty S, Cockburn IA, Kuk S, Overstreet MG, Sacci JB & Zafala F (2007). CD8⁺ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. *Nature medicine*, 13(9):1035-1041.
- Chan KH, Gu YL, Fai N, Park SP, Seto WH, Sham JST *et al.* (2003). EBV specific antibody-based and DNA-based assays in serologic diagnosis of nasopharyngeal carcinoma. *International Journal of Cancer*, **105**(5):706-709.
- Chan KT, Tam JS, Peiris JS & Seto WH (2001). Epstein-Barr virus infection in infancy. *Journal of Clinical Virology*, 21:57-62.
- Chattopadhyay PK, Chelimo K., Embury PB, Mulama DH, Sumba PO, Gostick, E *et al.* (2013). Holoendemic malaria exposure is associated with altered Epstein-Barr virus-specific CD8⁺ T-cell differentiation. *Journal of Virology*, 87(3), 1779-17788.
- Chêne A (2009). Impact of malaria on B-cell homeostasis and Epstein-Barr virus reactivation. Endemic Burkitt's lymphoma pathogenesis. Institutionen för mikrobiologi, tumör-och cellbiologi/Department of Microbiology, Tumor and Cell Biology.
- Chene A, Nyelen S, Donati D, Bejarano MT, Kironde F, Wahlgren M & Falk KI (2011). Effect of acute *Plasmodium falciparum* malaria on reactivation and shedding of the eight human herpes viruses. *Plos One*, 6(10): e26266.
- Choi HW, Breman JG, Teutsch SM, Liu S, Hightower AW, Sexton JD (1995) The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. *American journal of Tropical. Medicine and Hygiene*, 52:377–382.

- Chu-Qiong W & Jiman H (2016). Ubiquitous distribution of Epstein–Barr virus and the highly uneven distribution of nasopharyngeal carcinoma. *Chinese Medical Journal*, 129(20): 2506–2507.
- Condon LM, Cederberg LE, Rabinovitch MD, Liebo RV, Go JC, Delaney AS *et al.* (2014). Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. *Clinical Infectious Disease*, 59:501-508.
- Crawford DH (2001). Biology and disease associations of Epstein-Barr virus. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, 356(1408):461-73
- Damien GB, Djenontin A, Rogier C, Corbel V, Bangana SB, *et al.* (2010). Malaria infection and disease in an area with pyrethroid-resistant vectors in southern Benin. *Malaria Journal*, 9: 380.
- Daniel WW (1999). Biostatistics: A foundation for analysis in the health sciences. 7th edition. New York: John Wiley & sons.
- Danielle R & Glenda M (2016). Risk factors of malaria in children under the age of five years old in Uganda. *Malaria Journal*, 15:246.
- Dardari R, Menezes J, Drouet E, Joab I, Benider A, Bakkali H *et al.* (2008). Analyses of the prognostic significance of the Epstein-Barr virus transactivator ZEBRA protein and diagnostic value of its two synthetic peptides in nasopharyngeal carcinoma. *Journal of Clinical Virology*, 41(2): 96-103.
- de Thé G, Geser A, Day N.E *et al.* (1978). Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Uganda prospective study. *Nature* 274:756-61
- do Rosário AP, Lamb T, Spence P, Stephens R, Lang A, Roers A *et al.* (2012). IL-27 promotes IL-10 production by effector Th1 CD4⁺ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *The Journal of Immunology*, 188(3):1178-90.
- Dobbs KR & Dent AE (2016). *Plasmodium* malaria and antimalarial antibodies in the first year of life. *Parasitology*, 143(2):129-138.
- 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, series B Biological Science*, 356(1408):581-593.

- Dondorp AM, Lee SJ, Faiz MA, Mishra S, Price R, Tjitra E. *et al.* (2008). The relationship between age and the manifestations of and mortality associated with severe malaria. *Clinical Infectious Disease*, 47(2):151-7.
- DRG instruments GmbH, Germany. Epstein-Barr Virus (EBNA-1) IgG ELISA. Frauenberstr 18, D-35039. Division of DRG International
- Duah NO, Miles DJC, Whittle HC, Conway DJ (2010). Acquisition of antibody isotypes against *Plasmodium falciparum* blood stage antigens in a birth cohort. *Parasite Immunology*, 32:125–34.
- Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA & Trumpp A (2009). IFN alpha activates dormant haematopoietic stem cells in vivo. *Nature*. 458: 904–908.
- Fievet N, Moussa N, Tami G, Maubert M, Cot M, Deloron P & Chaouat G (2001). *Plasmodium falciparum* induces a TH1/TH2 disequilibrium, favouring the TH-1 type pathway in the human placenta. *Journal of Infectious Diseases*, 183:1530-1534.
- Francis EG (2010). History of the discovery of the malaria parasites and their vectors. Review. *Parasites & Vectors*, 3(1):5.
- Freitas do Rosario AP, Lamb T, Spence P, Stephens R, Lang A, Roers A *et al.* (2012). IL-27 promotes IL-10 production by effector Th1 CD4⁺ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *Journal of Immunology*, 188: 1178–1190.
- Fukuyama M, Hayashi Y, Iwasaki Y, Chong J, Ooba T, Takizawa T *et al.* (1994). Epstein-Barr virus associated gastric adenocarcinomas and Epstein-Barr virus infection of the stomach. *Laboratory Investigation*, 71: 73-81
- Genton B & D'Acremont V (2001). Clinical features of malaria in returning travelers and migrants. In: Schlagenhauf-Lawlor P, ed. Travelers— malaria. Hamilton ON :BC Decker Inc., 371–392
- Gething PW, Elyazar IR, Moyes CL, Katherine EB, Carlos AG, Anand PP *et al.* (2012). A long-neglected world malaria map: *Plasmodium vivax* endemicity in 2010. *PLoS Neglected Tropical Disease*, 6: e1814.
- Gething PW, Patil AP, Smith DL, Carlos AG, Iqbal RFE, Geoffrey LJ *et al.* (2011). A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malaria Journal*, 10: 378.

- 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.
- Haque T, Iliadou P, Hossain A, Crawford DH (1996) Seroepidemiological study of Epstein-Barr virus infection in Bangladesh. *Journal of Medical Virology*. 48: 17–21.
- Hassan A, Tasnim A, Tariq M, Syed F B, Muhammad UF & Niaz A. (2008). Parasite density and the spectrum of clinical illness in Falciparum malaria. *Journal of the College of Physicians and Surgeons*, 18 (6): 362-368.
- Henle W, Henle G, Andersson J, Ernberg I, Klein G, Horwitz CA *et al* (1986). Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proceedings of the National Academy of the Science USA*, 84:570-574.
- Hislop AD, Taylor GS, Sauce D & Rickinson AB (2007). Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annual Review in Immunology*, 25:587-617.
- Hjalgrim H, Friberg J & Melbye M (2007). The epidemiology of EBV and its association with malignant disease. In Arvin A, Campadelli-Fiume G, Mocarski E *et al.*, editors Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge University Press. pp. 929–959.
- Hughes DJ, Carol AD, Marie SS, Clare ES & Jeffery TS (2011). trans-Repression of Protein Expression Dependent on the Epstein-Barr Virus Promoter Wp during Latency. *Journal of Virology*, **85**(21):11435-11447.
- Hviid L, Barfod L, Fowkes FJ (2015). Trying to remember: immunological B cell memory to malaria. *Trends in Parasitology*, **31**(3):89-94.
- IARC (1997). Proceedings of the IARC Working Group on the evaluation of carcinogenic risks to humans. Epstein-Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8. IARC monographs on the evaluation of

- carcinogenic risks to humans / World Health Organization, *International Agency for Research on Cancer*, 70: 1-492.
- IARC (2012). (IARC Monographs on the evaluation of carcinogenic risks to Humans, No. 100B.) Epstein-barr virus. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK304353/>
- Ibrahim ID (2015). Effect of *Plasmodium falciparum* malaria on Epstein-Barr Virus (EBV). Reactivation during pregnancy and subsequent shedding of EBV in breast milk postpartum. *Maternal Child Health Journal*. 19:606–614
- Iwuafor AA, Egwuatu CC, Nnachi AU, Ita IO, Ogban GI, Akujobi CN *et al.* (2016). Malaria Parasitaemia and the use of insecticide-treated nets (INTs) for malaria control amongst under-5-year-old children in Calabar, Nigeria. *BMC Infectious Diseases*, 16: 151.
- Janz A, Oezel M, Kurzeder C, Mautner J, Pich D, Kost M, Hammerschmidt W & Delecluse HJ (2000) Infectious Epstein-Barr virus lacking major glycoprotein BLLF1 (gp350/220) demonstrates the existence of additional viral ligands. *Journal of Virology*. 74: 10142–10152
- Jayasooriya S, de Silva TI, Njie-jobe J, Sanyang C, Leese AM, Bell A.I *et al.* (2015). Early virological and immunological events in asymptomatic Epstein Barr virus infection in African children. *PLoS Pathogens*, 11(3): e1004746.
- Jeanette JR, Rosemary R, Peter OS, Dickens K, Mark LW& Moormann AM (2008). Family Environment Is Associated with Endemic Burkitt Lymphoma: A Population-based Case-control Study *The American Journal of Tropical Medicine and Hygiene*, 78(2); 338-343.
- Jenson HB (2004). Virologic Diagnosis, Viral Monitoring, and Treatment of Epstein-Barr Virus Infectious Mononucleosis. *Current Infectious Disease Report*. 6: 200–207.
- Jenson HB (2011). Epstein-Barr Virus. *Pediatrics in Review*, 32(9):375.
- Johanna ND, Marion P & Ian A.C. (2014). Antibody and B cell responses to *Plasmodium* sporozoites. *Froniers of Microbiology*, 5: 625.
- Kantele A & Jokiranta TS (2011). Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clinical Infectious Disease*. 52: 1356–62.

- Kume T, Oshima K, Shinohara T, Takeo H, Yamashita Y, Shirakusa T & Kikuchi M (1999). Low rate of apoptosis and overexpression of bcl-2 in Epstein-Barr virus-associated gastric carcinoma. *Histopathology*, 34: 502-509.
- Kwok H, Chan KW, Chan KH & Chiang AKS (2015) Distribution, persistence and interchange of Epstein-Barr Virus strains among PBMC, plasma and saliva of primary infection subjects. *PLoS ONE*, **10**(3): e0120710.
- Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J & Robets DJ. (2007). Malarial anaemia: of mice and men. *Blood*, 110: 18–28.
- Langhorne J, Francis MN, Anne-Marit S & Kevin M (2008). Immunity to malaria: more questions than answers. *Nature Immunology*, 9,:725–732.
- Lawrence MC, Laurel EC, Mark DR, Rhoda VL, Janice CG, Amanda SD *et al* (2014) Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: Effects of race/ethnicity and family environment. *Clinical Infectious Diseases*, 59(4):501–508
- Leanne JR, Marthe CD, Danielle IS, Jack T, Nicholas B, Jack SR *et al* (2009). Cellular tumor necrosis factor, gamma interferon and interleukin-6 responses as correlates of immunity and risk of clinical *Plasmodium falciparum* malaria in children from Papua New Guinea. *Infection and Immunity*, **77**(7):3033-3043.
- Leight ER & Sugden B (2001). EBNA-1: a protein pivotal to latent infection by Epstein-Barr virus. *Reviews in Medical Virology*. 10:83–100. [PubMed]
- Lengeler C (2004) Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database of Systematic Review: CD000363
- Li L, Liu D, Hutt-Fletcher L, Andrew M, Maria GM & Victor L (2002). Epstein-Barr virus inhibits the development of dendritic cells by promoting apoptosis of their monocyte precursors in the presence of granulocyte macrophage-colony-stimulating factor and interleukin-4. *Blood*, 99: 3725–3734.
- Lindsey M (2007). Epstein-Barr virus entry. *Journal of Virology*, **81**(15):7825-7832.
- Lucia M & Salvatore M (2002). The immune response to *plasmodium falciparum* malaria. *Lancet Infectious Disease*. 2: 472-78
- Maria W, Ingemar E, JieZhi Z, Jenny A & Erik A (2007). Epstein-Barr virus latency switch in human B-cells: a physico-chemical model. *BMC Systems Biology*, 1:40.
- Markus K & Wolfgang H (2012). Human B cells on their route to latent infection – Early but transient expression of lytic genes of Epstein-Barr virus. *European Journal of Cell Biology*, **91**(1):65-69.

- Matar CG, Anthony NR, O'Flaherty BM., Jacobs NT, Priyamvada L, Engwerda C.R *et al.* (2015). Gammaherpesvirus co-infection with malaria suppresses anti-parasitic humoral immunity. *Plos Pathogens*, **11**(5): e1004858.
- Mbulaiteye SM, Walters M, Engels EA, Bakaki PM, Ndugwa CM, Owor AM. & Biggar R.J (2006). High levels of Epstein-Barr virus DNA in saliva and peripheral blood from Ugandan mother-child pairs. *Journal of Infectious Diseases*, **193**(3), 422-426.
- McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Parmley RT *et al.* (1995). Association of Epstein-Barr virus with leiomyosarcomas in children with AIDS. *New England Journal of Medicine*, 332: 12-18.
- McKenzie FE, Prudhomme WA, Magill AJ, Forney JR *et al.* (2005) White blood cell counts and malaria. *The Journal of infectious diseases*, **192**(2):323-330.
- McNab F, Mayer-Barber K, Sher A, Wack A, & O'garra A. Type I interferons in infectious disease. *Nature Reviews Immunology*, **15**(2):87-103.
- Miller LH, Aikawa M & Dvorak JA (1975). Malaria (*Plasmodium knowlesi*) merozoites: immunity and the surface coat. *Journal of immunology*, 114:1237-1242.
- Monica C (1998). District laboratory practice in tropical countries (Part 1). Cambridge; Cambridge University Press. pp 255-260
- Moormann AM (2009). How might infant and paediatric immune responses influence malaria vaccine efficacy? *Parasite Immunology*, 31:547–559.
- Moormann AM, Chelimo K, Sumba PO, Lutzke M.L, Ploutz-synder R, Newton D *et al.* (2005). Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *Journal of Infectious Diseases*, **191**:1233–12388.
- Moormann AM, Chelimo K, Sumba PO, Tisch DJ, Rosemary R, James WK (2007). Exposure to holoendemic malaria results in suppression of Epstein-Barr virus specific T cell immunosurveillance in Kenyan children. *Journal of Infectious Disease*, 195: 799–808.
- 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.
- Moormann AM, Heller KN, Chelimo K, Embury P, Ploutz-Synder R, Juliana OT *et al.* (2009). Children with endemic Burkitt lymphoma are deficient in EBNA1-specific IFN-gamma T cell responses. *International Journal of Cancer*, 124: 1721–1726.

- Munn DH & Mellor AL (2013). Indoleamine 2, 3 dioxygenase and metabolic control of immune responses. *Trends in immunology*, 34: 137–143.
- Mutalima N, Molyneux E, Jaffe H, Kamiza S, Borgstein E., Nyengo M *et al.* (2008). Associations between Burkitt Lymphoma among children in Malawi and infection with HIV, EBV and Malaria: Results from a Case-Control Study. *PLoS ONE*, 3(6): e2505.
- Nicholas JW, Sasithon P, Tran TH, Abul Faiz M, Olugbenga AM & Arjen MD (2014). Malaria. *Lancet*, 383 (9918): 723-35.
- Njie R, Bell AI, Jia H, Croom-Carter D, Chaganti S, Hislop AD *et al.* (2009). The effects of acute malaria on Epstein-Barr virus (EBV) load and EBV-specific T cell immunity in Gambian children. *Journal of Infectious Diseases*, 199(1): 31-38.
- Oaks SC, Mitchell VS & Pearson GW (1991). Malaria: Obstacles and Opportunities. Washington (DC): National Academies Press (US); ISBN-10: 0-309-04527-4
- Offinso- North District Assembly. (2012). The composite budget of the Offinso- North District Assembly for the 2015 fiscal year. MTEF Budget document.
- Offinso- North District Assembly. (2015). The Composite Budget of the Offinso- North District Assembly for the 2015 fiscal year. MTEF Budget document.
- Olowe OA, Makanjuola OB, Awa AO & Olowe RA (2015). Malaria in Africa and the historical perspective: The Journey so Far. *Journal of Biology and Medical Sciences*, 3: 2409-5117.
- Oludare AO, Kristin AH & Henry HB (2011). Progress and problems in understanding and managing primary Epstein-Barr virus infections. *Clinical Microbiology. Review*, 24: 193-209.
- Osier HA, Gregory F, Spencer DP, Linda M, Federica V & Kevin KA *et al.* (2008). Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. *Infection and Immunity*, 76(5):2246-2248.
- Overstreet MG, Cockburn IA, Chen YC & Zavala F. Protective CD8+ T cells against Plasmodium liver stages: immunobiology of an ‘unnatural’ immune response. *Immunological Reviews*, 225(1):272-283.
- Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, Visintin A *et al.* (2007). Malaria hemozoin is immunologically inert but radically enhances innate responses by

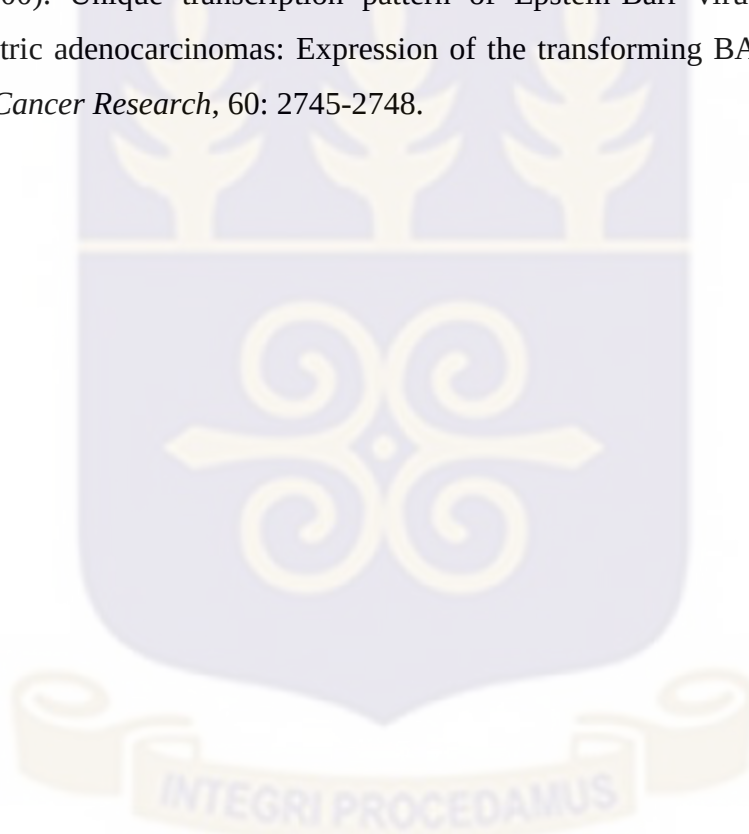
- presenting malaria DNA to Toll-like receptor 9. *Proceedings of National Academy Science United State of America*; 104: 1919–1924.
- Paschale M & Clerici P (2012). Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. *World Journal of virology*, **1**(1): 31–43.
- Paul ML (2013). Keeping it quiet: chromatin control of gammaherpesvirus latency. *Nature Reviews Microbiology*, 11: 863–875.
- Piriou E, Amolo SA, Sumba O, Fiore N, Middeldorp JM, Moormann AM *et al.* (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):906-13
- Poirier P, Doderer-Lang C, Atchade PS, Lemoine JP, de l'Isle MC, Abou-Bacar A *et al.* (2016). The hide and seek of *Plasmodium vivax* in West Africa: report from a large-scale study in Beninese asymptomatic subjects. *Malaria Journal*, **15**(1): 570.
- Pradhan V & Gosh K (2013). Immunological disturbances associated with malaria infection. *Journal of Parasitic Diseases*, .37: 11-15.
- Premier Medical Corporation Limited. 32-35, Shree Ganesh Indl. Estate, Kachigam, Nani Daman, Daman - 396 215. India. Website: www.premiermedcorp.com
- 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.
- Rainey JJ, Mwanda WO, Wairiumu P, Moormann AM, Wilson ML & Rochford R (2007). Spatial distribution of Burkitt's lymphoma in Kenya and association with malaria risk. *Tropical Medicine & International Health*, **12**(8):936–943.
- Raphael M, Audouin J, Lamine M, Delecluse HJ., Vuillaume M, Lenoir GM *et al.* (1994). Immunophenotypic and genotypic analysis of acquired immunodeficiency syndrome-related non-Hodgkin's lymphomas. Correlation with histologic features in 36 cases *American Journal of Clinical Pathology*, 101(6):773-82
- Rasti N, Falk KI, Donati D, Gyan BA, Goka BQ, Troye-Blomberg M *et al.* (2005). Circulating Epstein-barr virus in children living in malaria-endemic areas. *Scandinavian Journal of Immunology*, **61**(5):461-5.

- Rickinson AB & Kieff E (2001). Epstein-Barr virus. In: Field's virology. 4th edition. Philadelphia: Lippincott-Raven. pp. 2575–2627.
- Robert W S & Omumbo JA (2006). Disease and mortality in Sub-Saharan Africa. 2nd edition. National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA.
- Rochford R, Cannon MJ. & Moormann AM (2005). Endemic Burkitt's lymphoma: a polymicrobial disease. *Nature Reviews Microbiology*. 3:182–187. [PubMed: 15685227]
- Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P *et al.* (1991). Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. *American Journal of Tropical Medicine Hygiene* 45: 297–308.
- Sample J, Young L, Martin B, Chatman T, Kieff E & Rickinson A (1990). Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B and EBNA-3C genes. *Journal of Virology*. In: Encyclopedia of Virology, 64:4084-4092
- Saraiva M & O'Garra A (2010). The regulation of IL-10 production by immune cells. *Nature Review in Immunology*, 10: 170–181.
- Sato T, Onai N, Yoshihara H, Arai F, Suda T & Ohteki T (2009). Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. *Nature Medicine*. 15: 696–700.
- Shu CH, Chang YS, Liang CL, Liu ST, Lin CZ & Chang P (1992). Distribution of type A and type B EBV in normal individuals and patients with head and neck carcinomas in Taiwan. *Journal of Virology*, 38: 123–130.
- 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: 642–647.
- Silins SL, Sheritt MA, Silleri JM, Cross SM, Elliott SL, Bharadwaj M & Misko IS (2001). Asymptomatic primary Epstein-Barr virus infection occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. *Blood*, 98(13), 3739-3744.
- Simji SG, Akingdih MT, Ibrahim HO, Isa SE & Anejo-Okopi J (2017). Prevalence of malaria parasitaemia and its association with ABO blood group in Jos, Nigeria. *International Journal of Infectious Diseases and Therapy*, 2(3): 59-65.

- Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Theeraphap C, Maureen C *et al.* (2012). A global map of dominant malaria vectors. *Parasite & Vectors*, 5: 69.
- Slobedman B, Barry PA, Spencer JV, Avdic S & Abendroth A. Virus-encoded homologs of cellular interleukin-10 and their control of host immune function. *Journal of Virology*, **83**(19):9618-9629.
- Slyker JA, Casper C, Tapia k, Richardson R, Bunts L, Meei-Li H *et al.* (2013). Clinical and virologic manifestations of primary Epstein-Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. *Journal of Infectious Diseases*, **207**(12): 1798–1806.
- Smith CM, Gill MB, May JS & Stevenson PG (2007). Murine gammaherpesvirus-68 inhibits antigen presentation by dendritic cells. *PLoS One*, 2: e1048
- Speck SH & Ganem D (2010). Viral latency and its regulation: lessons from the gamma-herpesviruses. *Cell Host & Microbe*, **8**(1):100-15.
- 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>
- Sugden B (2014) Epstein-Barr Virus: The Path from association to causality for a ubiquitous human pathogen. *PLoS Biology* **12**(9): e1001939.
- Thompson MP & Kurzrock R (2004). Epstein-Barr virus and cancer (2004). *Clinical Cancer Research*, **10**(3), 803-821.
- Tierney RJ, Edwards RH, Sitki-Green D, Croom-Carter D, Roy S, Yao QY, Raab-Traub N & Rickinson AB (2006). Multiple Epstein-Barr virus strains in patients with infectious mononucleosis: comparison of ex vivo samples with in vitro isolates by use of heteroduplex tracking assays. *Journal of Infectious Diseases*, **193**(2):287-297
- Tiwawech D, Srivatanakul P, Karaluk A & Ishida T (2003). Significance of plasma IgA and IgG antibodies to Epstein-Barr virus early and viral capsid antigens in Thai nasopharyngeal carcinoma. *Asian Pacific Journal of Cancer*, 4(2):113-8.
- Tsuji M & Zarvala F. (2003). T cells as mediators of protective immunity against liver stages of plasmodium. *Trends in Parasitology*, 19:88-93.
- Virgin HW & Speck SH (1999). Unraveling immunity to gamma-herpesviruses: a new model for understanding the role of immunity in chronic virus infection. *Current Opinion in Immunology*, 11:371–379

- Warrell DA, Hemingway J, Marsh K, Sinden RE, Butcher GA & Snow RW (2012). Malaria. In: Oxford Textbook of Medicine (Warrell DA, Cox TM, & Firth JD. eds). Printed from Oxford Medicine online (www.oxfordmedicine.com). Oxford University Press, 2015.
- Wedderburn N, Davies DR, Mitchell GH, Desgranges C & de Thé G (1988). Glomerulonephritis in common marmosets infected with *Plasmodium brasilianum* and Epstein-Barr virus. *Journal of Infectious Diseases*, 158(4):789-94
- West PA, Protopopoff N, Rowland M, Cumming E, Rand A *et al.* (2013) Malaria risk factors in North West Tanzania: The Effect of spraying, nets and wealth. *PLoS ONE* .8(6): e65787
- WHO (2014). Severe Malaria. *Tropical Medicine and International Health*. 19: 7–131. <http://doi.org/10.1111/tmi.12313>
- Wilcox CR, Holder B & Jones CE (2017). Factors affecting the FcRn-mediated transplacental transfer of antibodies and implications for vaccination in pregnancy. *Frontiers in immunology*, 8:1294.
- World Health Organisation (2005). Malaria fact sheet. Retrieved from http://www.who.int/malaria/publications/world_malaria_report/en
- World Health Organisation (2015). Malaria. Retrieved from http://www.who.int/malaria/publications/world_malaria_report/en
- World Health Organization (1990). Severe and complicated malaria. *Transactions of the Royal Society of Tropical and Medical Hygiene*. 84(suppl 2): S1–S65
- World Health Organization (2000). Severe falciparum malaria. *Transactions of the Royal Society of Tropical and Medical Hygiene*, 94 (suppl 1):S1–S90.
- World Health Organization (2012). Factsheet: World malaria Report 2012. Retrieved from http://www.who.int/malaria/publications/world_malaria_report/en
- World Health Organization (2016). Factsheet: World malaria Report 2016. Retrieved from http://www.who.int/malaria/publications/world_malaria_report/en
- Wu MS, Shun CT, Wu CC, Hsu TY, Lin MT, Chang MC & Wang HP, Lin JT (2000). Epstein-Barr virus-associated gastric carcinomas: relation to *H. pylori* infection and genetic alterations. *Gastroenterology*, 118: 1031-1038
- Wu X, Gowda NM, Kumar S & Gowda DC (2010). Protein-DNA complex is the exclusive malaria parasite component that activates dendritic cells and triggers innate immune responses. *Journal of Immunology*. 184: 4338–4348.

- Young LS & Rickinson AB (2004). Epstein-Barr virus: 40 years on. *Nature Review*, 4(10), 757-768
- Young LS, Arrand JR & Murray PG (2007). EBV gene expression and regulation. In: Arvin A, Campadelli-Fiume G, Mocarski E, *et al.*, editors. Human herpesviruses: Biology, therapy, and immunoprophylaxis. Cambridge: Cambridge University Press. Chapter 27. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK47431/>.
- Zotos D & Tarlinton DM. (2012). Determining germinal centre B cell fate. *Trends in Immunology*, 33 281–288
- zur Hausen A, Brink A, Craanen M, Middeldorp JM, Meijer CJ, van den Brule AJ. (2000). Unique transcription pattern of Epstein-Barr virus in EBV-carrying gastric adenocarcinomas: Expression of the transforming BARTF1 gene. *Journal of Cancer Research*, 60: 2745-2748.



APPENDIXES

Appendix I:

INFORMATION SHEET

TITLE OF STUDY: A study of the correlation between Epstein-Barr Virus infection and the severity of malaria in Ghanaian children under 5 years of age

Principal Investigator: Bashiru Swabira

Department: Medical Laboratory Sciences

Phone: 0269682869

Purpose of Study

You are being asked to voluntarily make your child take part in this research study. But before you decide to allow your ward to participate in the study, it is important that you understand why the research is being carried out and what it entails. Please listen/read carefully and ask anything that is not clear to you or anything you need more information on.

The purpose of this study is therefore to find out if EBV in malaria children is the reason why these children have severe malaria compared to their counterparts who have malaria but not EBV.

Study Procedure

In order to know if your child has malaria, we have to withdraw at least 150ul of blood from his/her finger or heel for the diagnosis. IF it is positive, we would set out to look for the quantity of parasitaemia. More so, with the same blood, we would test for EBV and if it is also positive we would determine antibody titre using ELISA. We would take

the blood only once and the blood would be analysed at the Virology department of School of Biomedical and Allied Health Science (SBAHS).

Risk

There is only a minimal risk associated with drawing of blood from children which may include a little bleeding, pain, bruising and, rarely, infection. All of these are rarely common. You are assured that experts would be the ones to withdraw the blood from your ward and therefore make it as painless as possible.

Benefit

We will be testing for Epstein-Barr virus in your child which is normally not done in our various medical laboratories and it is therefore a benefit. It is important to know if your child has EBV and it will tell us if it is the reason his/her malaria is severe. Another potential benefit of your ward taking part in this study is that, very important information of your ward sickness will be generated without any cost. These findings will inform your Doctor on how best to go about his/her treatment without delay. Note that you will not receive any financial compensation in this study.

Voluntary Participation:

Allowing participation of your child in this research study is entirely voluntary. Refusal of participation is not going to alter the maximum attention that is going to be given to him/her with regards to his/her health. It would also not incur any penalty at all or loss of benefits.

However, should you decide to allow your ward be recruited in the study, you are also helping in the diagnosis of malaria and findings of this research would attract more attention on EBV and the need for its treatments in children.

Confidentiality

If you consent to let your ward be recruited in the study, you are assured that all health information pertaining to your child would be kept confidential. We would use numbers rather than names on any sample collected from your ward. When publishing the findings, the name of your ward would not appear in it.

Should you need more information, you should not hesitate to contact my supervisors as well.

Dr Alexander Martin-Odoom

Dr. Charles Brown



INFORMED CONSENT FORM

To be completed by the study participant's parent/guardian

Consent to participate in Research:

The nature and the purpose of the above research study have been openly explained to me in a language that I understand. I have also been made known the benefit and possible risk that is involved in the study. I have also been made aware that the entire study is entirely voluntarily and I can withdraw my ward at any time with/without any reason without attracting any penalty or being denied maximum treatment for my child.

Signing below therefore indicates that I have been duly informed about the research study in which I voluntarily agree to my child's participation. I have asked questions to clear any doubt and the information given to me has permitted me to make an informed and free decision about my child's participation in the study. By signing this consent form, I do not forgo any legal rights against the investigators and I can also withdraw from this study at any time. A copy of this consent form will be provided to me.

Name of guardian/parent-----

Signature/ thumbprint of Parent_____ **Date**_____



Appendix 2

Structured Questionnaire for interviews for the determination of risk factors of severe malaria

Q1. Demography of children

	Participant code	
	Gender	Male: Female
AGE	0-7months	
	8-23months	
	24-48months	
	Age>48months	
	<60months	
	Study site	

Q3. Clinical data

Fever ()	Malaise ()
vomiting ()	Convulsion ()
Diarrhoea ()	Respiratory distress ()
Pallor ()	

Q2. Indicators (Laboratory Findings) of Severe Malaria

Laboratory findings	Quantity
Parasitaemia (parasites/ul)	
Haemoglobin($10^6/l$)	
White blood cells ($10^9/l$)	
Red blood cell($10^{12}/l$)	

Risk Factors of Underlying Disease

Factors		Yes	No
Duration of illness	1-2 days		
	3-4days		
	5-more days		
Use of protective measures	ITNs		