ASSOCIATION OF THE ABO BLOOD GROUPS WITH FALCIPARUM MALARIA IN THE MALARIA ENDEMIC COUNTY OF BUSIA IN WESTERN KENYA.

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DECLARATION

This dissertation is my original work and it has not been presented for a degree or any other award in any other University or Institution.

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…….

DEDICATION

Lovingly dedicated to my daughter Zara.

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The ultimate gratitude goes to God almighty for seeing me through my academic pursuits.

ABSTRACT

Background

The interplay between malaria parasites and blood group antigens remains a fascinating subject with potential to contribute to the development of new interventions to reduce the global burden of malaria. Elucidation of the association between the erythrocyte ABO antigens status and infection with *Plasmodium falciparum* can bring about understanding of the differences noted in the ABO blood group variation in malaria endemic areas.

Objective

To determine the ABO distribution among patients and its association with malaria infection in the malaria endemic county of Busia in western Kenya.

Hypothesis

The study hypothesized that during infection with *P falciparum*, group O offers a survival advantage, group A confers a disadvantage, and group B has an intermediate.

Design

This was a cross-sectional study that sequentially enrolled patients who were suspected to have malaria, and had their blood tested for the malaria parasite and the ABO blood group status.

Study site

The Busia county referral hospital

Method

Upon giving their consent, a total of 246 febrile patients who were clinically examined and suspected to have malaria had their blood tested for the malaria parasite by making thick and

thin blood smears and observing under the microscope at 100x. Parasitaemia was determined for patients to tested positive for *P.falciparum*. The patients' blood groups were determined using the tube technique. The variables used in the study questionnaire were age, gender, ABO blood group status, malaria blood slide examination result and clinical diagnosis of the study subjects. Data entry was done in MS excel, database management and analysis was done using Statistical Package for Social and Sciences (SPSS) version 13 software.

Results

Of the 246 respondents, 63.8% of them were aged below 5 years of age and 28.9% were above 16 years. 60.5% of the respondents were females. Both age (X^2=0.997 p>0.05) and sex (X^2=0.975 p>0.05) were however not significantly associated with disease status. 44.31% (109) of the respondents were blood group O, 26% (64) were blood A and 24.4% (60) were blood group B. Only 5.3% (13) were blood group AB. More than one thirds 37.8% (93) of the respondents were MPs positive. All the respondents had less than 1000 parasites /μ L of blood. Blood group was not statistically associated (X^2=2.857, p=0.827) with parasitaemia. Malaria infection showed significant association with blood group A (X^2=4.736, p<0.05), B (X^2=0.570, p<0.015) and AB (X^2=2.751, p<0.05). Blood group O was not statistically associated with getting malaria infection(X^2=0.005, p>0.05).

Conclusion

During infection with *P.falciparum*, blood group O individuals are conferred with a protective advantage, group A individuals are at a disadvantage, while blood group B has an intermediate effect.

Table of Contents

ABBREVIATIONS:

CD: Cluster of Differentiation

CR1: Complement receptor 1

CSA: Chondrotinsulphate A

DNA: Deoxyribonucleic acid

FUT1: fucosyltransferase 1

Gal: Galactose

GalNAc: N-acetylgalactosamine

ICAM: Intercellular Adhesion Molecule

MOH: Ministry of Health

PfEMP1: *Plasmodium falciparum* Erythrocyte Membrane Protein 1

RBCs: Red blood cells

SPSS: Statistical Package for the Social Sciences

TSP: Thrombospondin

VCAM: Vascular cell adhesion molecule

WHO: World Health Organization

Bs: Blood slide

MPs: Malaria parasites

LIST OF TABLES:

LIST OF FIGURES:

1.0 BACKGROUND OF THE STUDY

In 2013, 584,000 people globally died from malaria. 90% of the deaths occurred in Sub-Saharan Africa where *Plasmodium falciparum* is the most prevalent of the malaria parasites and the leading cause of malaria deaths (World Malaria Report 2014). *P.falciparum* has been called "the strongest known force for evolutionary selection in the recent history of the human genome" (Kwiatkowski, 2005). The signature of *P.falciparum* has been its enormous toll on human life, especially children where untreated children have a 20-fold higher fatality rate than adults. Malaria, like other infectious diseases that kill children selects for survival genes and effectively prevents transmission of genotypes unfavorable to survival. One such gene that is selected for by malaria is the *ABO* blood group gene which has three alleles namely, A, B and O, coding for different types of agglutinogens attached to the surface of red blood cells (RBCs) and hence determining an individual's blood group.

The plasmodium parasite has established a close relationship between itself and the RBCs. In fact, the severe pathophysiological manifestations of malaria caused by *Plasmodium falciparum* are a direct consequence of the parasite's blood stage replication cycle, during which merozoites repeatedly invade, multiply within, and destroy RBCs. Consequently, RBCs have evolved specific receptor-ligand interactions, some of which involve the ABO blood group antigens, to facilitate their adherence and invasion by merozoites. Therefore, any variation in the erythrocyte ABO antigens can change the penetration and establishment of the parasite in the merozoites.

In terms of severity, *P.falciparum* is the most severe of the human malarial parasites. Central to its pathophysiology is the production and release of an adhesive ligand known as the *Plasmodium*

falciparum erythrocyte membrane protein 1 (PfEMP1) to the surface of infected RBCs. PfEMP1 consequently facilitates the adherence of the infected erythrocytes to a range of host cells, such as endothelial cells (cytoadherence), uninfected erythrocytes (rosetting) and platelets (platelet-mediated clumping). This leads to the sequestration of infected erythrocytes into the microvasculature of various organs thereby causing obstruction to their microcirculation. Consequently, ischemia develops with resultant tissue hypoxia, particularly in the brain, kidneys, lungs and gastrointestinal tract. Hypoglycemia and lactic acidosis are other complications characteristic of severe malaria. In cases where sequestration occurs in the brain, cerebral malaria develops. The sequestration of infected erythrocytes in the microvasculature of the host's internal organs causes most of the morbidity and mortality in *falciparum* malaria. Sequestration also favors the development of the parasite by protecting it from the filtering action of the spleen. Various host receptors on the surface of uninfected RBCs, including the ABO blood group antigens have been implicated in rosette formation (Carlson et al.,
1992). It has therefore seemed possible that the ABO blood group system may be linked to susceptibility or protection from severe malaria.

There is strong epidemiological evidence that the ABO phenotype may modulate disease severity and outcome of *P. falciparum* malaria, with blood groups A and B associated with increased disease severity compared to blood group O. Clinical studies conducted in Thailand and East Africa proved the effect of ABO blood groups in rosetting by demonstrating that the frequency of rosetting parasites in blood isolated from group O patients was less than in blood isolated from patients with blood groups A, B and AB (Barragan et al., 2000). Other studies have also reported that

P.falciparum forms rosettes with group O RBCs with lesser frequency compared with group A and B (Herrera et al., 2009). In addition, the rosettes formed with group O RBCs have been reported to be smaller and more easily disrupted than those formed in groups A, B and AB erythrocytes (Ong et al., 2011). Also, the plasmodium parasite has been observed to have a reduced capacity to invade group O erythrocytes (Millet et al., 1977; Perkins,

1981; Breuer et al., 1983; Friedman et al., 1984) while macrophages targeting *P.falciparum* infected erythrocytes have been shown to clear infected O erythrocytes more avidly than infected A and B erythrocytes (Wolofsky et al., 2012).This could indicate some resistance of group O to the severe presentation of malaria.

It is therefore hypothesized that during infection with *P falciparum*, group O offers a protective and survival advantage, group A confers a disadvantage, and group B has an intermediate effect thereby subjecting the ABO blood group system to malaria related selection pressure. This association is consistent with the observed predominance of blood group O in malaria endemic sub-Saharan Africa relative to other parts of the world where malaria is not endemic (Cserti et al., 2007).

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Malaria is a life-threatening blood disease caused by the Plasmodium parasite that is transmitted to humans by the Anopheles mosquito. Said to be one of the world's oldest diseases, malaria has often decimated populations with greater efficiency than wars. The disease remains endemic in 97 countries and territories around the world leaving an estimated 3.3 billion people at risk of contracting it. In 2013 only, 198 million cases of malaria occurred globally, leading to 584,000 deaths, 78% of which were in children under 5 years of age (World Malaria Report, 2014).

The WHO African region bears the heaviest malaria burden, where an estimated 90% of all malaria deaths occur (World Malaria Report, 2014). This is largely because the majority of the malaria infections in Africa are caused by *Plasmodium falciparum* which is the most dangerous of the human malarial parasites as it is responsible for most of the mortality and morbidity associated with the disease. Also, the parasite has a high rate of replication, resulting in high levels of parasitaemia. The high burden of malaria in Africa may also be attributed to the fact that the most effective malaria vector, the mosquito Anopheles gambiae, is the most widespread in Africa and the most difficult to control (Uneke,

2008). In addition, the warm and humid African climate allows malaria transmission to occur all year round while malaria control efforts are cumbered by the lack of resources and the poor socio-economic conditions.

As in its Sub-Saharan neighbours, Malaria remains a leading cause of morbidity and mortality in Kenya, where it is estimated that 28 million people are at risk of contracting malaria (Kenya Malaria Operational Plan, 2015). In 2012, there were more than 9 million cases and about 30,000

deaths from malaria in the country thereby accounting for 1 in every 20 malaria-caused deaths worldwide. Also, 3000 Kenyan children under the age of five years die of malaria annually (WHO). This accounts for 20% of all deaths among children under 5 years in the country (MOH 2006).

2.2 LIFE CYCLE OF *Plasmodium falciparum*

The Plasmodium species exhibits three life-cycle stages in the human host. The sporozoites, merozoites and the gametocytes. During a blood meal, a female *Anopheles* mosquito inoculates sporozoites into the blood stream of its human host. The sporozoites travel to the liver and infect the liver cells where in a period of 5-16 days, they grow, divide and produce tens of thousands of haploid forms called merozoites, per liver cell. The liver cells then rupture to release these merozoites into the bloodstream where they infect erythrocytes. Inside erythrocytes, the parasite undergoes a 48-hour-long developmental process that starts with the ring stage (0–24 hours), followed by DNA replication and parasite growth during the trophozoite stage (24–36 hours) and, ultimately, the schizont stage (36–48 hours), during which infectious merozoites are formed. These merozoites are released through the rupture of the infected erythrocytes and repeat the cycle by invading more erythrocytes. Each cycle consisting of erythrocyte rupture to release merozoites and the subsequent invasion of more erythrocytes by the released merozoites causes fever in the patient.

A small percentage of merozoites leave the cycle of asexual multiplication and instead undergo differentiation into the sexual forms of the parasite known as gametocytes, , which then circulate in the bloodstream and will eventually be ingested by a mosquito during a blood meal. Gametocytes will further develop into mature sex cells called gametes and undergo fertilization in

the gut of the mosquito to form a zygote. The zygote elongates and becomes motile to form an ookinete which penetrates the midgut wall of the mosquito and develops into oocysts. The oocysts grow and rupture to release sporozoites which make their way to the mosquito's salivary glands and await inoculation into a human host during the mosquito's next blood meal. The cycle of human infection begins again when the mosquito bites another person (Fig. 1)

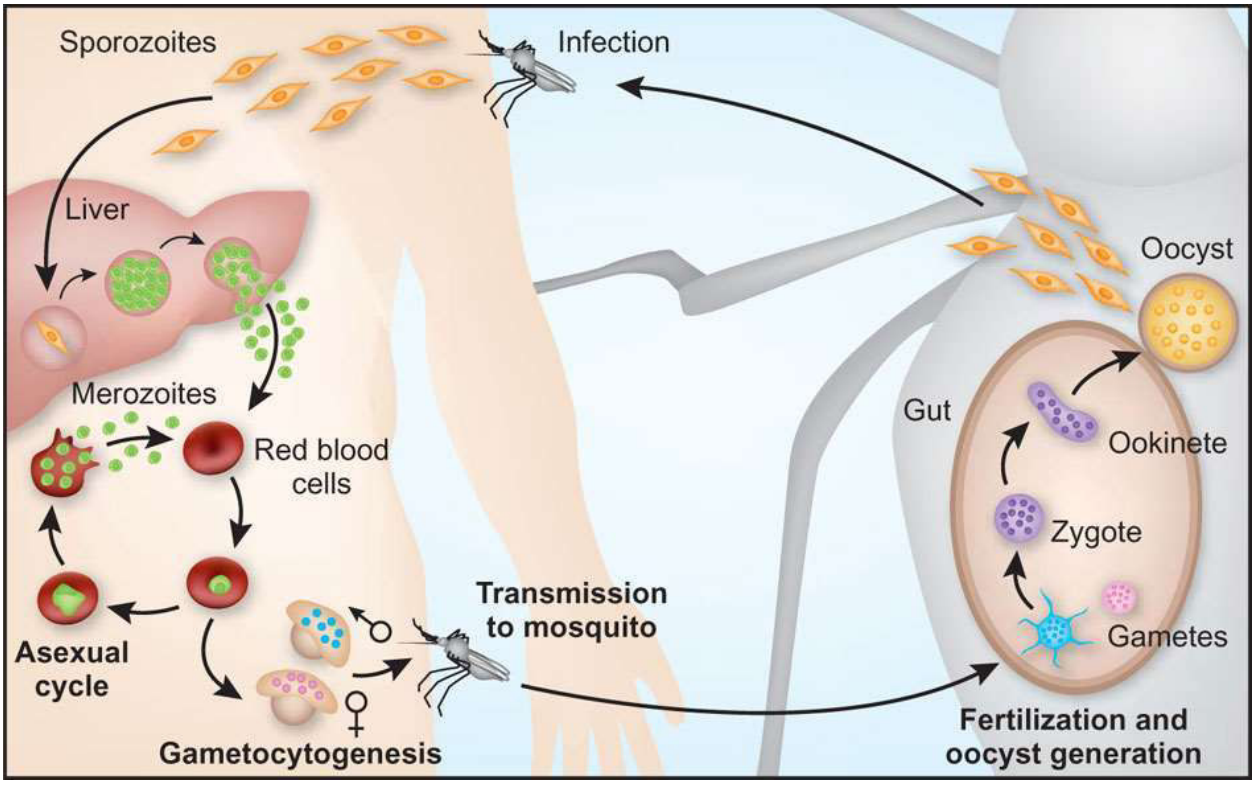

Figure 1: Life cycle of *Plasmodium falciparum* (Pasvol, 2010)

2.3 PATHOGENESIS OF *Plasmodium falciparum*

The pathogenesis of human *P falciparum* infection is a complex interplay of parasite-induced RBC alterations (Maier et al., 2009) and microcirculatory abnormalities (Grau et al., 2003),accompanied by

local and systemic immune reactions, resulting in multiple clinical forms of variable severity (Marsh et al., 1995).

Immediately after invasion of RBCs, the *P.falciparum* parasite begins to make significant alterations to the structure of the erythrocyte so as to facilitate the movement of nutrients into, and waste products and parasite-derived proteins out of the cell to meet the needs of the growing parasite. A tubovesicular membrane network extending from the parasite vacuole membrane probably has a central role in the transport processes. The parasite also extensively modifies the membrane of the host cell resulting in changes in permeability, morphology, deformability and adhesive properties of the host erythrocyte (Miller et al., 2002).The erythrocytes become stiffer after infection, generally reflecting changes in the structure of the membrane cytoskeleton (Cooke et al., 2001; Nash et al., 1989).

One of the most striking structural alterations on the membrane of the host cell is the formation of electron-dense knobs-like protrusions, which are composed of parasite-expressed proteins, such as the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (Cooke et al., 2001). The expression of PfEMP1 on the surface of Infected RBCs (iRBCs) is central to the pathogenesis of *P.falciparum.* The extracellular portion of this protein contains distinct domains by virtue of which it interacts with receptors such as Thrombospondin (TSP), CD36, ICAM-1, VCAM, E-selectin, chondroitin sulphate A (CSA), CD31 and P-selectin (Newbold et al., 1999) (Fig.2) on endothelial cells (cytoadherence) and complement receptor1 (CR1), heparin-sulphate-like molecules and A or B blood group antigens on uninfected RBCs (rosetting). PfEMP1 also has the capacity to bind platelets (platelet-mediated clumping).

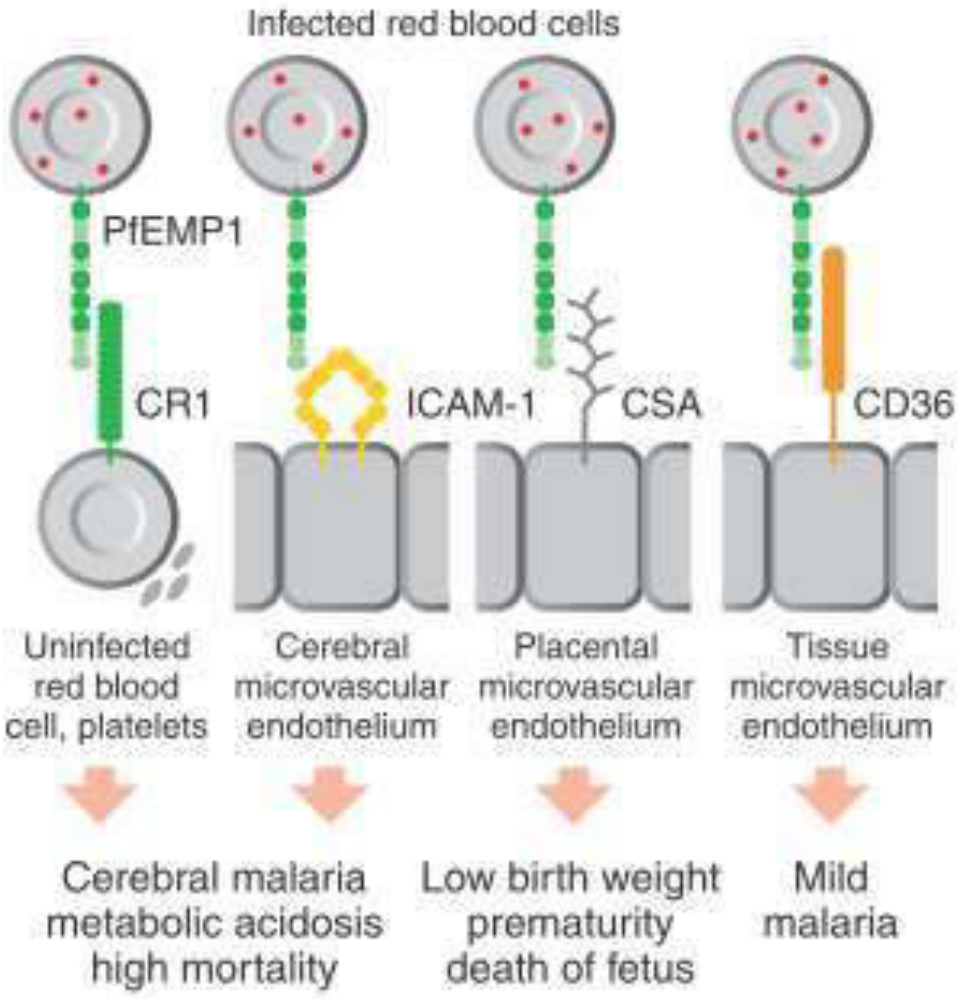

Figure 2: Different variants of PfEMP1 bind to adhesion proteins expressed on different endothelia and lead to different disease phenotypes (Penman and Gupta, 2008)

Rosetting and platelet-mediated clumping are thought to be accompanied by adhesion to endothelial cells (Kaul et al., 1991). Therefore, cytoadherence, rosetting and platelet-mediated clumping collectively facilitate the sequestration of iRBCs in the microvasculature of various organs (Fig.3) and tissues such as the heart, brain, lungs, muscle and adipose tissue. Consequently, ischemia develops with resultant tissue hypoxia. Sequestration also allows the iRBCs to escape retention and destruction by the spleen, thereby enhancing parasite growth, leading to high

parasitemia.

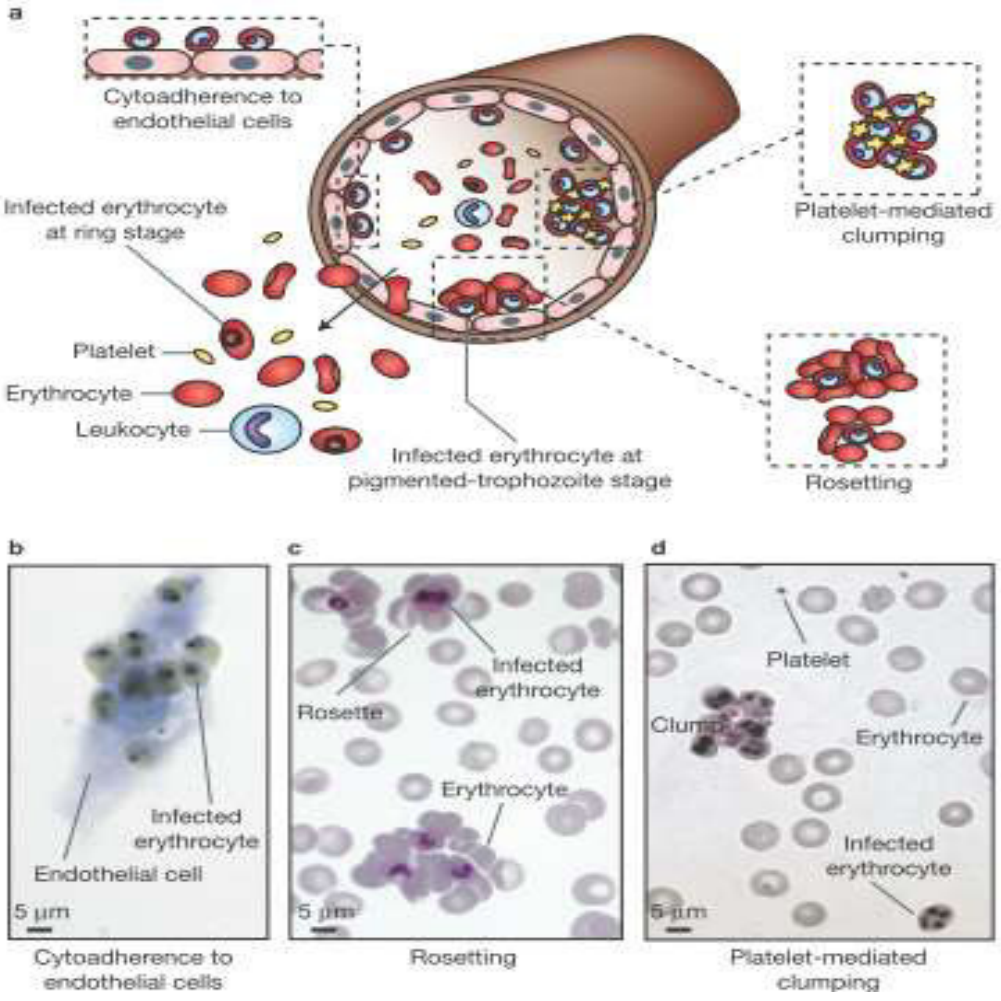

Figure 3: Adhesion of erythrocytes infected with *Plasmodium falciparum* to human cells (Rowe et al., 2009)

2.4 THE ABO BLOOD GROUP ANTIGENS

Host genetic factors modulate the risk and severity of malaria infection via specific mediators, which can be different in various epidemiological settings (Adam et al., 2007). RBC invasion by *P.falciparum* merozoites and the adhesion of parasitized RBCs to other cells play a central role to *falciparum* malaria pathophysiology. Cell surface glycans such as the ABO blood group antigens and

other related antigens could modulate some of those specific cell interactions (Cserti et al., 2007).

Discovered in 1900 by Austrian scientist and Nobel Laureate Karl Landsteiner, the antigens of the ABO system are oligosaccharide that are attached to the proteins and lipids on the surface of RBCs. These antigens are synthesized stepwise by the action of glycosyltransferase enzymes encoded for by the ABO gene locus located on chromosome 9 at 9q34.1-q34.2. The gene has three alleles: A, B and O. For the synthesis of the A and B antigens to occur, a precursor O antigen (also referred to as the H antigen) must be present. The O antigen, which is synthesized by an enzyme encoded for the by the H (FUT1) locus in RBCs is in the forms of —Lipid—Glucose—Galactose—N-acetylglucosamine—Galactose—Fucose. The A allele of the ABO locus codes for N-acetylgalactosamine (GalNAc) transferase which catalyzes the formation of an α-1,3 glycosidic bond between the outermost galactose component of the O antigen and GalNAc. Meanwhile the B allele encoded the synthesis of Gal transferase which glycosidically adds an extra galactose to the O antigen at the α-1.3 position. The O allele however encodes an enzyme with no function, and therefore neither A or B antigen is produced, leaving the underlying precursor (the H antigen) unchanged

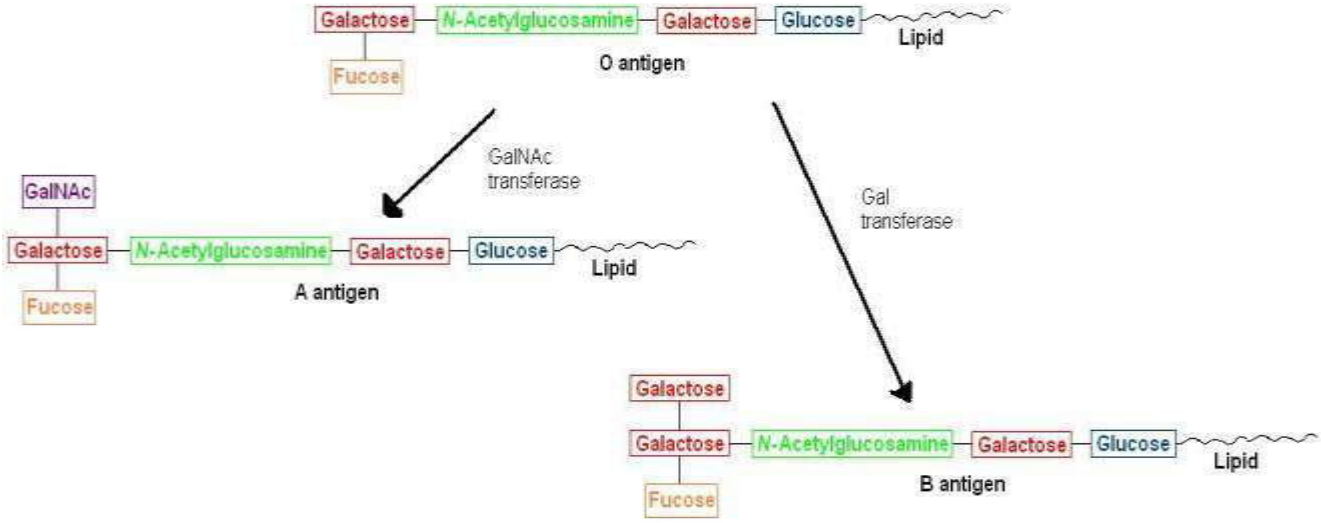

Figure 4: Structures of A, B, and O Oligosaccharide Antigens. Abbreviations: Gal, galactose; GalNAc, N-acetylgalactosamine.

2.5 ASSOCIATION BETWEEN ABO BLOOD GROUP SYSTEM AND *FALCIPARUM* MALARIA.

Various studies have sought to establish an association between the ABO blood types and malaria. These studies have however been unable to establish an unequivocal link between the ABO blood groups and the prevalence and incidence of malaria parasitaemia. A study conducted at a tertiary care hospital at Navi, Mumbai, India found that people with blood group O are more prone to malarial infection in endemic areas (Singh et al., 2015), while another conducted among inhabitants of Odoakpu area of Onitsha South Local Government Area in Anambra state in Nigeria reported malaria to be most prevalent in individuals with blood group AB (Ilozumba et al., 2009). This assertion that individuals with certain blood groups were predisposed to *P.falciparum* malaria infected was however not supported by a study that prevalence of malaria parasitaemia and the

predisposition of the ABO blood groups to *falciparum* malaria among blood donors at a Ghanaian hospital (Muntaka et al., 2013; Opoku-Okrah et al., 2010).

Clinical reports of ABO blood groups and *P.falciparum* infection, however reveal a clear correlation between disease severity and ABO groups, with blood group A being associated with increased disease severity and blood group O being associated with decreased disease severity thereby conferring a survival advantage, whereas blood group B has an intermediate effect.

In a study of 489 patients with *P.falciparum* malaria at the Sanyati Baptist Hospital in Kadoma, Zimbabwe, coma was found to be 3-times more common among group A individuals compared with non-A persons (9 of 104 group A versus 11 of 385 with non-A blood, 7.0; *p* .008; odds ratio, 3.6) (Fischer et al., 1998), thereby confirming the hypothesis that group A blood group correlates with disease severity.

In Ethiopia, Teketse et al assessed210 cases of *falciparum* malaria (70 severe and 140 uncomplicated) compared with 190 cases of healthy controls in the malaria endemic localities of Awash, Metehara and Ziway. Severe malaria was defined as having at least one of the severe malaria syndromes (cerebral malaria, severe anemia and circulatory collapse). Results showed that in the severe malaria category, there were 25 (35.7%), 15 (21.4%), 14 (20%) and 16 (22.9%) blood group A, B, AB and O patients, respectively. Blood group O was found to be the dominant blood type in both uncomplicated malaria (45.7%) and healthy controls (41.6%). Also, a case of severe malaria was almost twice as likely to be of type A as to be of type O (odds ratio (OR) 0.42, 95% confidence interval (CI) 0.20-0.88, P = 0.019), and more than twice as likely to be of type B as to be of type O (OR 0.38, 95% CI 0.16-0.89, P = 0.02). Furthermore, individuals with severe malaria

were about six fold less likely to be of O as to be of type AB (OR 0.19, 95% CI 0.07-0.51, P = 0.0005). The study therefore revealed that patients with blood group O, had a reduced chance of developing severe *falciparum* malaria as compared to patients with other blood groups (Teketse et al., 2010).

A similar study was conducted in India, of 100 malaria infected patients of which 63 cases were positive for *P.falciparum* and 37 cases were positive for *P.vivax* infection and 11 patients had mixed infection. Determination of blood groups showed that 22 were blood group A, 42 B, 35 O and 1 was AB. When the clinical courses between different groups were compared using the following parameters for severe infection—a parasitic load of >10/1000 RBCs, severe anemia with hemoglobin < 6 g%, platelet count of <10,000/mm3, hepatomegaly or splenomegaly or clinical signs of severe malaria such as fever >101ºF and other organ involvement, it was observed that 'O' group had an advantage over other the groups (Deepa et al.,2011)

Contrary to the hypothesis that blood group O confers a protective advantage from severe malaria, a study conducted by Herrera et al in Apartado, Colombia, on 92 patients of which 49 had severe malaria and 43 uncomplicated malaria found that severe malaria was more frequent among patients classified with blood group O (65.3 %). However, this association was not statistically significant (Herrera et al.,2009).

Other similar studies (Zerihun et al.,2011; Panda et al., 2011) agree with the hypothesis that while individuals with blood groups A,B and AB are more susceptible to severe malaria with blood group A being the most prone, individuals with blood group O have a protective advantage.

3.0 RATIONALE OF STUDY

The association of the ABO blood groups and falciparum malaria has been demonstrated in various populations.

However, there are no studies on the direct association between the ABO blood groups and *P.falciparum* malaria infection and severity that have been done in Kenya. Therefore, this gap necessitates a study to investigate the association between the ABO blood groups and *P.falciparum* malaria infection in a Kenyan population.

4.0 OBJECTIVES

4.1 BROAD OBJECTIVE

To assess the distribution of the ABO and Rhesus D blood groups and their relationship with *P.falciparum* malaria among febrile patients who sought medical attention at the Busia, Siaya and Homabay county referral hospitals in western Kenya.

4.2 SPECIFIC OBJECTIVES.

1. To determine the ABO and Rhesus D blood groups' frequency distribution among febrile patients attending the Busia, Siaya and Homabay county referral hospitals in October 2015.

2. To use both clinical and laboratory diagnosis to determine whether febrile patients attending the county referral hospitals in Busia, Homabay and Siaya in October 2015 are infected with P.falciparum

3. Determine if there is a significant association of the ABO blood groups with malaria infection.

5.0 MATERIALS AND METHODS

5.1 Study design: This was a cross-sectional study that used purposive sampling to sequentially enroll patients diagnosed to have malaria and typed their blood groups in order to assess the association of ABO blood status and malaria infection.

5.2 Study area: The study was done in the malaria endemic county of Busia located in the Lake Victoria malaria endemic region. Most parts of Busia County fall within the Lake Victoria Basin. The altitude is undulating and rises from about 1,130m above sea level at the shores of Lake Victoria to a maximum of about 1,500m in the Samia and North Teso Hills. The average temperature is 22°C and the rainfall amount ranges between 750mm and 1,800mm per annum. The county has a malaria parasite prevalence that is estimated to be equal to or greater than 20% (Kenya Malaria Operational Plan, 2015). The perennial transmission of malaria in the county of Busia is as a collective result of the low altitude, high temperatures, high humidity and proximity to the lake which provides the ideal breeding site and thereby maintains high numbers of the anopheles mosquito which is the malaria vector (KEMRI 2015). The vector life cycle is usually short with high survival rate due to the suitable climatic conditions and the annual entomological inoculation rates range between 30 and 100. The site was therefore selected based on malaria endemicity which was sought to obtain rich data.

5.3 Study population: The study population was composed of malaria suspected, febrile patients of all ages, who visited the Busia county referral hospital in October 2015.

5.4 Selection criteria:

- **Exclusion criteria:** Patients whose blood was not tested for malaria and febrile patients who had taken any antimalarial drugs within the last two weeks before the blood test. Patients who did not consent or assent to the study were also excluded.
- **Inclusion criteria:** Patients who were suspected to have malaria and tested for malaria using thick and thin blood smear and microscopy.

5.5 Clinical and Laboratory diagnosis

Before collecting the blood sample, explanation about the study was given and a written informed consent forms were given to adults over 18 years and the guardians of children under 6 years of age while assent forms were given to children between the ages of 6-18 years. Capillary blood was collected by finger pricking using 70% isopropanol and sterile disposable lancet. Heel puncture was used for infants. Immediately, thin film was spread on grease free, frosted end, labeled slide using a smooth edged slide spreader. Thick film was also prepared on the same slide. Thin film was then fixed with methanol. The blood film was stained with 10% Giemsa for 10 minutes. Finally, the films were examined under an oil immersion microscope objective (100x). Parasitaemia was determined for febrile patients who tested positive *for P. falciparum* by counting the number of parasites (asexual forms only) against 200 white blood cells (WBC). This counting was done by using hand tally counters. Then, the number of parasites per microliter of blood was calculated.

The blood group of the study participants was determined using the tube technique.

5.6 Sample size calculation:

Sample size was estimated using the formula recommended by Cochran (1963)

$$n = \frac{(Z_{1-\alpha/2})^2 (p)(1-p)}{(d)^2}$$

where

n = the required sample size

$Z_{1-\alpha/2}$ = Standard Normal Deviation which is equal to 1.96 corresponding to 95% confidence interval

p = Prevalence of the issue under study

d= Confidence limit of the prevalence (p) at 95% confidence interval 1-0.95 = 0.05

Therefore:-

- $Z_{1-\alpha/2}$ will be 1.96 since it's the standard normal variate at 5% level of significance
- P will be 0.20 since currently the prevalence is 20% (Kenya Malaria Operational Plan, 2015)
- d= the margin of error will be 0.05

Sample Size= $\frac{(1.96)^2(0.2)\ (1-0.2)}{(0.05)^2}$

n =245.86

A sample size of 246 patients was therefore used.

5.7 Data Analysis plan.

Data was entered in Microsoft Excel, checked for its correctness, and exported to and analyzed using SPSS version 13. Chi-square test was used to assess the difference between frequencies (the associations between blood groups and *P. falciparum* malaria cases). ANOVA was used to test the difference between parasitaemia means. Observed difference was considered to be significant for $P<0.05$.

6.0 ETHICAL CONSIDERATIONS

Ethical clearance for this study was obtained from the Kenyatta National Hospital/University of Nairobi Ethical Review Committee (KNH/UON-ERC).

7.0 RESULTS

7.1 Socio-demographic characteristics of the respondents

A total of 246 questionnaires were distributed and completely filled. Almost two thirds (63.8%) of the respondents were aged below five years and more than a quarter (28.9%) were above sixteen years **(Figure 5).**

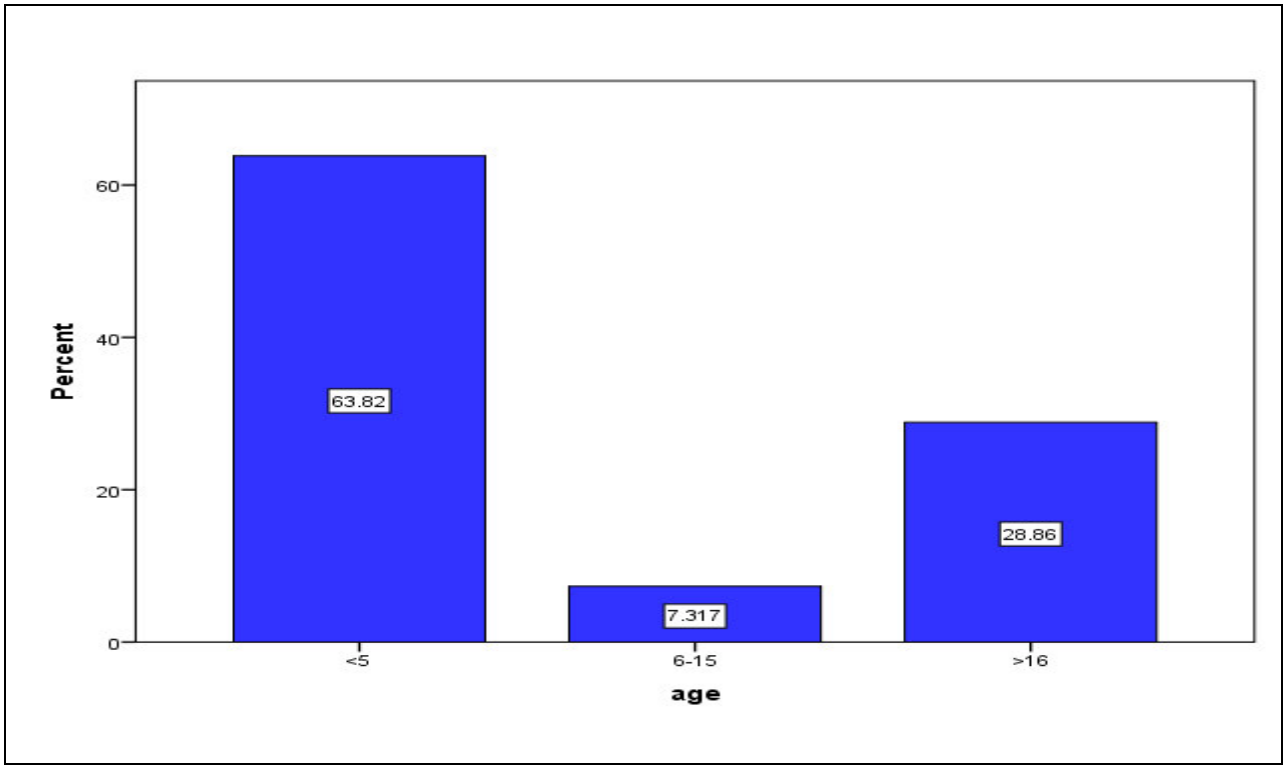

Figure 5: Distribution of age of the respondents in Busia county October 2015

More than half 60.5% (149) of the respondents were females **(Figure 6).** Three quarters (74%) of the respondents were from Matayos sub-county, while almost half 40.7% (100) of the respondents were from township village **(Table 1).**

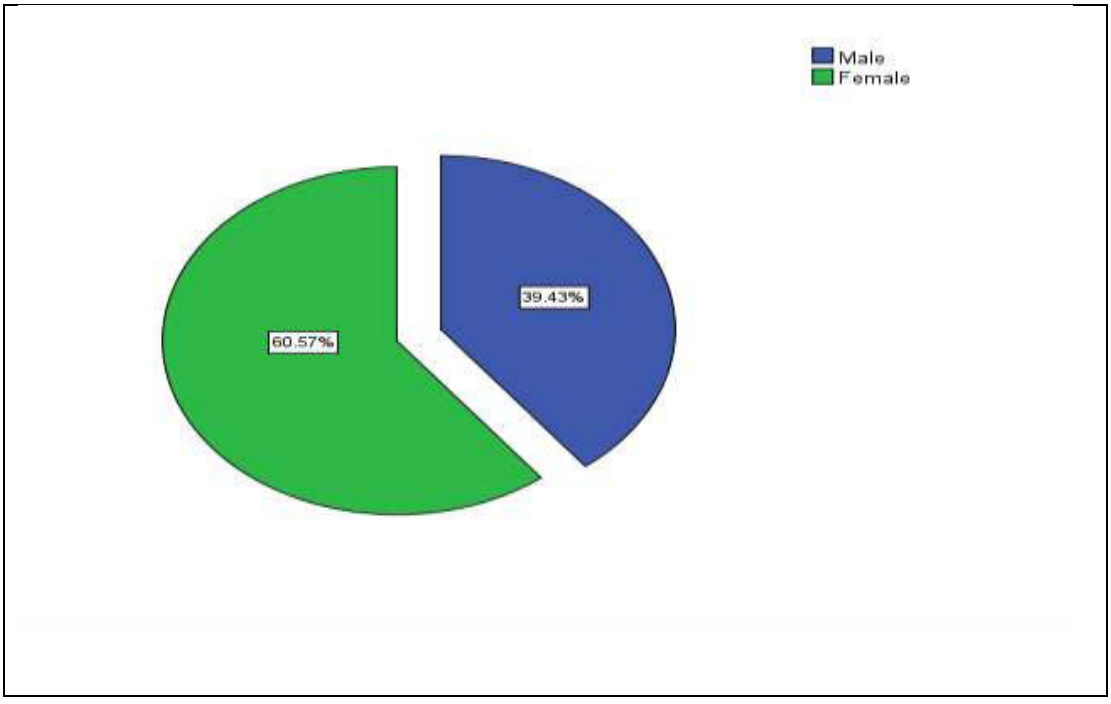

Figure 6: Distribution of gender of the respondents in Busia County October 2015

Variable	Frequency	Percentage	Confidence interval	
			Lower	Upper
Sub County				
Matayos	182	74	67.9	79.7
Nambale	9	3.7	1.6	6.5
Butula	9	3.7	1.6	6.1
Bunyala	4	1.6	0.4	3.7
Teso South	40	16.3	11.4	20.7
Samia	2	0.8	0.0	2.0
Village				
Agoloto	39	15.9	11.4	20.7
Bugengi	11	4.5	2.0	7.7
Nambale township	4	1.6	0.4	3.3
Maenje	16	6.5	3.3	9.8
Township	100	40.7	34.6	47.1
Esikulu	34	13.8	9.8	18.3
Emulanda	1	0.4	0.0	1.6
Mundika	11	4.5	2.0	6.9
Aterait apegal	2	0.8	0.0	2.0
Sikingi	4	1.6	0.4	3.6
Lugala	1	0.4	0.0	1.2
kisoko	1	0.4	0.0	1.2
Marachi east	14	5.7	2.8	8.9
Sigalame	3	1.2	0.0	2.8
mauko	1	0.4	0.0	1.2
Mundebi	3	1.2	0.0	2.8
Ochudo	1	0.4	0.0	1.2

Table 1: Distribution of Sub County and village of residence of the respondents in Busia County 2015

7.2 Provisional diagnosis of the respondents

A quarter (25.2%) of the respondents had headache, 6.9% had chills and rigors, 11.8 % were experiencing joint pains, 14.2 % had diarrhoea and 17.5% were vomiting. More than half (54.9%) of the respondents had fever. 18.3% of them were experiencing general malaise. Only 6.9% of the respondents had loss of appetite **(Table 2).** Upon clinical diagnosis**,** 93.5% of the respondents were recommended for malaria parasite testing, 2.4% were recommended for the widal test for typhoid and 4.1% were recommended for both widal and malaria parasite testing **(Table 3).** However all 246 patients were tested for malaria.

Variable	**Present (Frequency)**	**Percentage**	**Absent (Frequency)**	**Percentage**
Symptoms				
Headache	62	25.2	184	74.5
Chills and rigors	17	6.9	229	93.1
Joint pains	29	11.8	217	88.2
Diarrhoea	35	14.2	211	85.8
Vomiting	43	17.5	203	82.5
Fever	135	54.9	111	45.1
Malaise	45	18.3	201	81.7
Loss of Appetite	17	6.9	229	93.1

Table 2: Distribution of symptoms of the respondents in Busia County October 2015

Variable	Frequency	Percentage	Confidence interval	
			Lower	Upper
Clinical diagnosis				
Bs for MPs	230	93.5	90.2	96.3
Widal	6	2.4	0.8	4.5
Bs for Mps and Widal	10	4.1	2.0	6.5

Table 3: Distribution of clinical diagnosis of respondents at Busia County October 2015

7.3 ABO blood group among the respondents in Busia County October 2015

Almost half (44.31%) of the respondents were blood group O. A quarter (26%) were blood A and 24.4% were blood group B. Only 5.3% of the respondents were blood group AB **(Figure 7).**

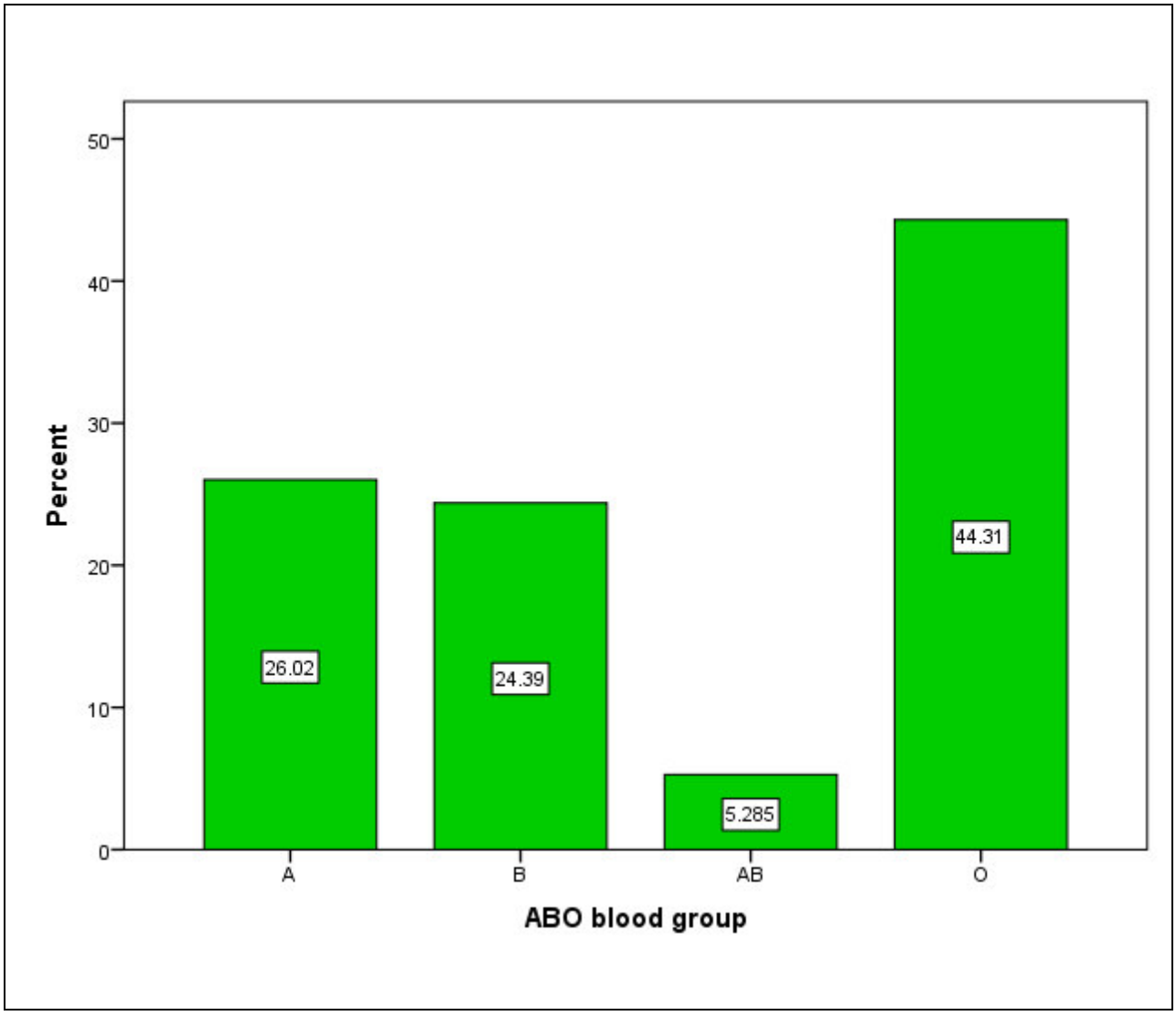

Figure 7: Distribution ABO blood group among the respondents in Busia County October 2015

7.4 Malaria diagnostic results of the respondents in Busia County October 2015

More than one thirds 37.8% (93) of the respondents were MPs positive **(Figure 8).** All the respondents had less than 1000 parasites /µ L of blood **(Table 4).**

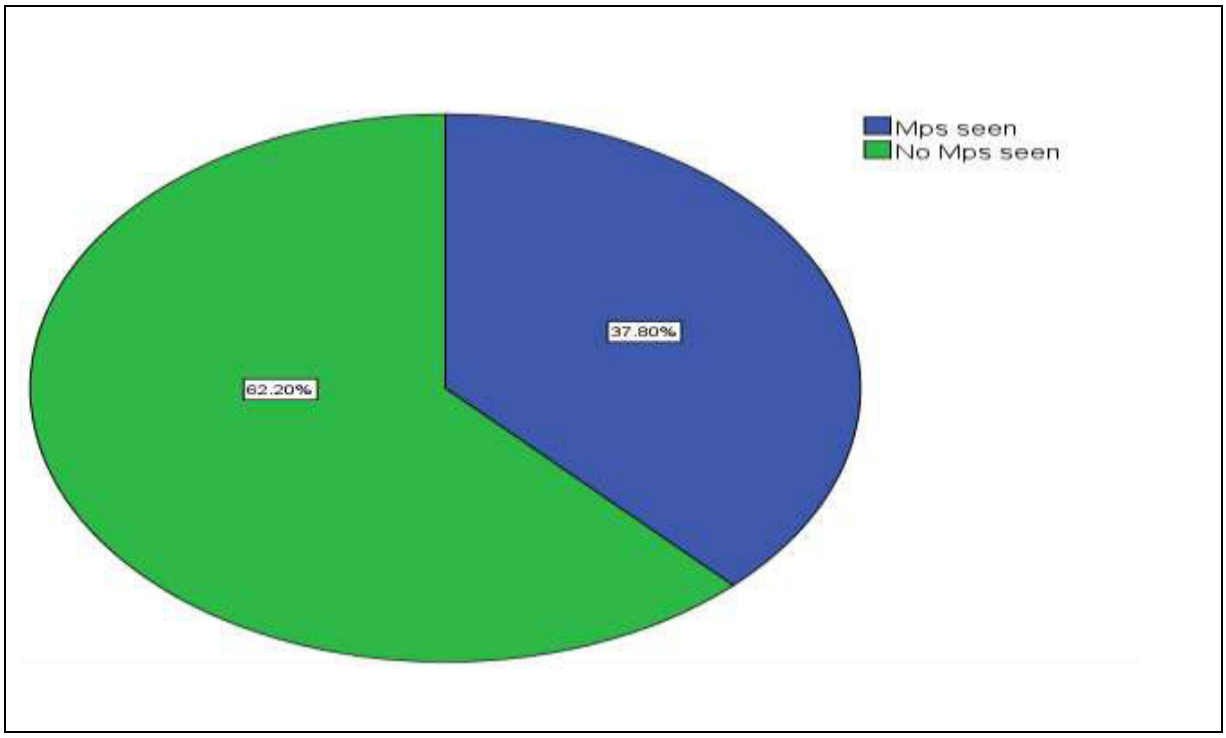

Figure 8: Distribution Malaria diagnostic results among the respondents in Busia County October 2015

Variable	Frequency	Percentage	Confidence interval	
			Lower	Upper
Parasitaemia				
<200 parasites /µL of blood	70	75.3	65.6	83.9
201-600 parasites/ µL of blood	17	18.3	10.8	26.9
601-999 parasites /µL of blood	6	6.5	2.2	11.8

Table 4: Distribution of parasitaemia of respondents at Busia County October 2015

7.5 BIVARIATE ANALYSIS

7.5.1 Association between socio demographics Age, sex and disease status

The chi-square test of association used in bivariate analysis to find association between disease status and socio demographic variable age and sex. Both sex and age were not significantly associated with disease status (X^2=0.997 p>0.05) and (X^2=0.975 p>0.05) respectively **(Table 5).** It was also found out that males were 0.765 (95% CI 0.44-1.335) times less likely to be infected with *P. falciparum*

Age (years)	**Number examined**	***P.falciparum* positive**	***P.falciparum* negative**
≤ 5	157	63 (40)	94 (60)
6-15	18	6 (33.3)	12 (66.7)
>16	71	24 (33.8)	47 (66.2)
Total	246	93 (37.8)	153 (62.2)
χ2, P	0.997, 0.607		
Sex			
Male	97	33 (34)	64 (66)
Female	149	60 (40.2)	89 (59.8)
Total	246	93 (37.8)	153 (62.20
χ2, P	0.975, 0.348		

Table 5: Prevalence of malaria by age and sex among the study participants at the county referral hospitals in the malaria endemic county of Busia, October 2015

7.5.2 Association between ABO blood group and disease status

All febrile patients examined for malaria were also tested for ABO blood groups. In general malaria infection showed significant association with blood group (X^2=4.828, p<0.05). The highest proportion of infection 44% was observed among respondents with blood group A followed by those with blood group O 39.4%. Malaria infection showed significant association with blood group A (X^2=4.736, p<0.05), B (X^2=0.570, p<0.015) and AB (X^2=2.751, p<0.05). Blood group O was not statistically associated with getting malaria infection(X^2=0.005, p>0.05).

Blood group	**Number examined**	***P.falciparum* positive**	***P.falciparum* negative**	**χ2, P**
A	64	29 (44.6)	35 (55.4)	4.736, 0.042
B	60	16 (26)	44 (74)	0.570, 0.015
AB	13	5 (38)	8 (62)	2.751, 0.023
O	109	43 (39.4)	66 (60.6)	0.005, 0.058
Total	246	93 (37.8)	153(62.2)	4.828, 0.025

Table 6: **Frequency of ABO blood groups among febrile patients at the county referral hospital in Busia in October 2015**

7.5.3 Association of Blood group, age and diseases status

Blood group and age were subjected to bivariate analysis with diseases status using chi-square test of association. According to the results *P. falciparum* did not show association with age in all the 4 blood groups **(Table 7).**

Blood group	**Age group**	**Numbers with blood type**	***P.falciparum* positive**	***P.falciparum* negative**	***χ2, P***
A	**<5**	43	21 (48.2)	22 (51.8)	
	5-15	7	3 (42.8)	4 (57.2)	
	>15	14	5 (35.7)	9 (64.3)	
	Total	64	29 (45.3)	35 (54.7)	0.753, 0.686
B	**<5**	42	12 (28.5)	30 (71.5)	
	5-15	6	1 (16.6)	5 (83.4)	
	>15	12	3 (25)	9 (75)	
	Total	60	16 (26.6)	43 (73.4)	0.402, 0.818
AB	**<5**	7	3 (42.8)	4 (47.2)	
	5-15	0	0 (0)	0 (0)	
	>15	6	2 (33.3)	4 (66.7)	
	Total	13	5 (38.4)	8 (61.6)	0.124, 0.725
O	**<5**	65	27 (41.5)	38 (58.5)	
	5-15	5	2 (40)	3 (60)	
	>15	39	14 (35.8)	25 (64.2)	
	Total	109	43 (39.4)	66 (60.6)	0.325, 0850

Table 7: Comparison of frequency of ABO blood groups among *P. falciparum*-infected individuals with that of non-Plasmodium-infected individuals at Busia County referral Hospital, October 2015

7.5.4: Association of blood group and parasitaemia

About 65.5% (19/29), 87.5% (14/16), 80% (4/5) and 76.7% (33/43) of those infected individuals of blood group A,B, AB and O respectively had parasite density of less than 200 parasite/ µL of blood. In contrast 7% (2/29), 6.25 (1/16) and 4.7 (2/43) *P. falciparum* infected individuals of blood group A, B and O respectively had parasite density ranging between 601-999 parasites / µL of blood. Blood group was not statistically associated (X2=2.857, p=0.827) with parasitaemia **(Table 8).**

Variable	**Parasitaemia**			**Total**	**χ2, P**
	<200 parasites /µL of blood	601-999 parasites /µL of blood	601-999 parasites /µL of blood		
Blood group					2.857, 0.827
A	19 (65.5)	8 (27.5)	2 (7)	29	
B	14 (87.5)	1 (6.25)	1 (6.25)	16	
AB	4 (80)	1 (20)	0 (0)	5	
O	33 (76.7)	8 (18.6)	2 (4.7)	43	

Table 8: Association of blood group with parasitaemia

8.0 DISCUSSION

This study has demonstrated that the blood groups in the ABO system vary in malaria infection. Indeed malaria has placed the strongest known selective pressure on the human genome since the origination of agriculture within the past 10,000 years, and, has been the driving force behind some haematological diseases such as sickle-cell anemia, thalassemia and glucose-6-phosphatase deficiency. According to the "malaria hypothesis" proposed by Haldane in 1948, these diseases arose from polymorphisms in the erythrocyte DNA to confer resistance to malaria, with heterozytes having a balance between the benefits of resistance to malaria and the detrimental effects of the diseases. Consequently, these diseases are found to be highly prevalent in African populations where *falciparum* malaria endemic.

Similarly, polymorphisms in the ABO gene are subjected to selective pressure by *falciparum* malaria. Several studies have found that during infection with *P.falciparum*, blood group O offers a protective advantage, group A confers a disadvantage while group B has an intermediate effect. The disadvantage conferred on the blood group A antigen by malaria is believed to have driven its mutation to the group O antigen which is identical to the group A allele for the first 261 nucleotides, at which point a guanosine base is deleted, resulting in a frame-shift mutation which produces a premature stop codon and failure to produce a functional A or B transferase. This mutation decreases susceptibility to malaria infection and severity of clinical disease, thereby favoring survival from malaria.

The selective pressure exerted on the ABO gene by malaria resultantly influences the global distribution of the ABO blood groups with a high prevalence of group O coupled with a low prevalence of group A phenotypes being observed in tropical regions where malaria is rampant. An

especially high prevalence of group O and low prevalence of group A is found throughout sub-Saharan Africa where *P. falciparum* imposes its highest burden. In the Western hemisphere, the distribution of group A and group O generally matches malaria's tropical distribution. From the tropical regions of Central and South America southward, the indigenous peoples are almost exclusively group O. In Asia, the prevalence of group O rises among peoples who live closer to the equator. For example, in Beijing, China (a cold weather zone) group O is 29% and group A 27%, but in Canton, China (a more tropical zone) group O is 46% and group A is 23%. In contrast, group A is the predominant blood group in the colder regions of the Earth where malaria has not been endemic (Cserti and Dzik, 2007). This is consistent with the findings of this study which found that the highest percentage of the study participants had the O blood group phenotype (44.3%) followed by blood group phenotypes A (26%), B (24.4%) and AB (5.3%). Blood group B seems to have an intermediate effect as proposed in the hypothesis.

Malaria infection showed significant association with blood group A (X^2=4.736, $p<0.05$), B (X^2=0.570, $p<0.015$) and AB (X^2=2.751, $p<0.05$). Blood group O was not statistically associated with getting malaria infection(X^2=0.005, $p>0.05$). This is also consistent with previous reports suggesting that individuals with blood groups A, B and AB are more susceptible to *P.falciparum* infection than those with O blood group

Possible mechanisms relating to these associations include molecular mimicry of the A and B antigens by *P.falciparum*, selective preference of the A antigen as a receptor for merozoite penetration of RBCs, increased macrophage-mediated phagocytosis of *P.falciparum* infected group O erythrocytes and the formation of larger and stronger rosettes by group A erythrocytes.

The malaria parasite as well as other infectious agents have been shown to mimic the ABO system antigens as an immune evasion technique (Athreya and Coriell, 1967). *Plasmodium* has in fact been shown to possess the group A antigens (Oliver- Gonzalez and Torregrossa, 1944). Further evidence on the mimicry of the A and B antigens by *P.falciparum* was given by a study that showed an increase of anti-A and anti-B titres in the blood group O phenotype individuals exposed to malaria (Kano et al., 1968), with higher titres being observed in individuals that live in malaria endemic areas or had suffered several attacks (Druilhe et al., 1983; Oliver-Gonzalez and Torregrossa, 1944). The *plasmodium* parasite may therefore be better tolerated by individuals that have the A and B blood group antigens, thereby increase susceptibility to infection and development of clinical disease. In contract, individuals with the O phenotype, especially in the malaria endemic areas are less likely to suffer an infection as they possess antibodies against the A and B antigens that are also found on the *plasmodium* parasite.

The ABO antigens have also been implicated in the invasion process of merozoites into the RBCs, with some of the molecules involved in the different phases of invasion being associated to some extent with the ABO phenotypes (Loscartales et al., 2007). It has in fact been shown that during erythrocyte invasion, *P.falciparum* significantly prefers blood group A erythrocytes (Chung et al., 2005), thereby increasing susceptibility of blood group A individuals to infection by *P.falciparum*. This may justify the high proportion of malaria positive individuals of blood group A in the present study.

P.falciparum infected group O erythrocytes are also more efficiently cleared by macrophages than infected A and B erythrocytes. This was shown in a study where human macrophages in vitro and mouse monocytes in vivo phagocytosed *P.falciparum* infected O erythrocytes more avidly than A and B infected erythrocytes. The enzymatic conversion of B erythrocytes to type O erythrocytes

before infection also significantly enhanced their uptake by macrophages to levels comparable to those with infected O wild-type erythrocytes. This study not only showed that the ABO blood group antigens influence macrophage clearance of *P.falciparum* infected erythrocytes but also suggested an additional mechanism by which blood group O may confer resistance to malaria (Wolofsky et al., 2012).

Several other studies have established that parasitized erythrocytes form rosettes more readily with RBCs of blood groups A, B, or AB than with blood group O RBCs, with parasite-triggered RBC rosette formation being associated with increased severity of clinical disease (Uneke, 2007; Udomsangpetch et al., 1993; Rowe et al., 1995; Pathirana et al., 2005). Rosette formation is governed by strong adhesive forces, with lectin-like bindings between parasite-derived proteins exposed on the surface of *P.falciparum* infected RBCs and various carbohydrate moieties present on the uninfected erythrocyte. The strongest carbohydrate receptors have been shown to be contained within the blood group A and B antigens (Carlson, 1993), thereby justifying the ease with which group A and B infected erythrocyte form rosettes, and accounting for the formation of larger and stronger rosettes by the non-O blood groups (Carlson et al., 1992; Udomsangpetch et al., 1993). Being stronger, the rosettes formed by the group A and B *P.falciparum* infected erythrocytes are less easily disrupted than those formed by group O erythrocytes. This allows the group A and B parasitized cells to avoid the host's normal splenic clearance mechanisms that remove aged or damaged erythrocytes (Mebius et al., 2005). Consequently, parasitized group A and B erythrocytes persists longer in the body, allowing multiplication of the *plasmodium* parasite, thereby predisposing individuals with blood groups to clinical disease.

9.0 CONCLUSION

The findings of this study showed that blood group O most prevalent in the malaria endemic county of Busia. It also established a significant association between the ABO blood groups with *falciparum* malaria, where individuals of blood group O were found less likely to be infected with *P.falciparum*, whereas individuals of blood group A were found more likely to have malaria. Blood group B appeared to confer an intermediate effect. The findings of this study therefore agreed with the proposed hypothesis that during an infection with *P.falciparum*, blood group O offers a survival advantage, group A offers a disadvantage, and group B has an intermediate effect.

10.0 STUDY LIMITATIONS

The study was initially meant to cover a wider geographical scope by also covering the malaria endemic counties of Siaya and Homabay in Western Kenya, but was unable to due to financial and time constraints.

11.0 RECOMMENDATIONS

Further in-depth studies are required to clearly assess and establish the role of the ABO blood group antigens in *falciparum* malaria. This may open a new insight into the glycobiology of infectious diseases and contribute to a wider understanding of malaria pathogenesis. Based on the findings of this study, special attention may be required to be given to individuals of blood groups A and B in the management and control of malaria.

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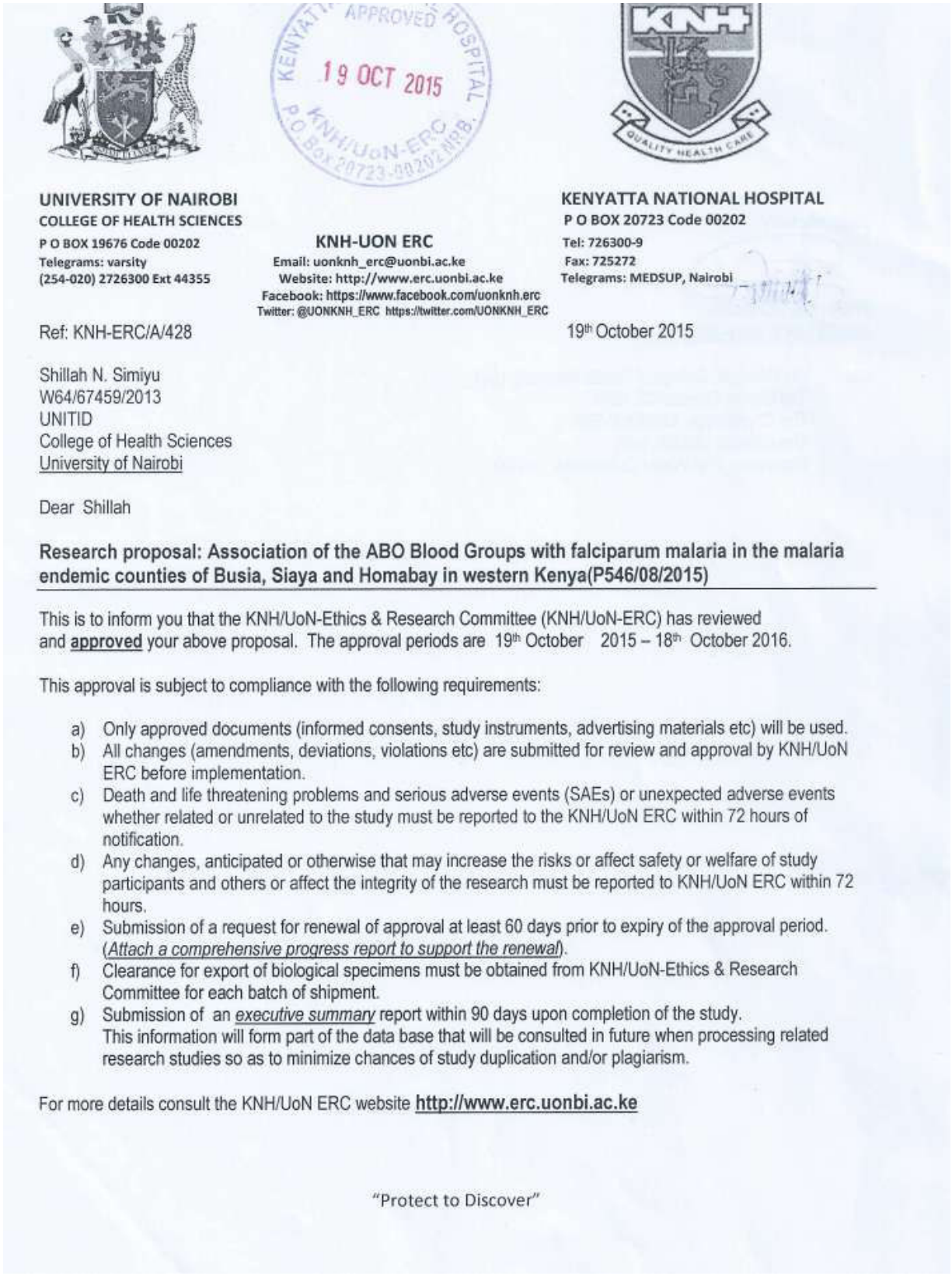
APPENDICES
APPENDIX 1: KNH-UON ERC LETTER OF ETHICAL APPROVAL



APPROVED
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19th October 2015

Shillah N. Simiyu
W64/67459/2013
UNITID
College of Health Sciences
University of Nairobi

Dear Shillah

Research proposal: Association of the ABO Blood Groups with falciparum malaria in the malaria endemic counties of Busia, Siaya and Homabay in western Kenya(P546/08/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 19th October 2015 – 18th October 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **http://www.erc.uonbi.ac.ke**

"Protect to Discover"

APPENDIX 2: Information & Consent/Assent Form

University of Nairobi Institute of Tropical and Infectious Diseases
Investigator: Ms. Shillah Simiyu
Supervisors: Prof.Dr. Walter Mwanda

STUDY TITLE: Association of the ABO blood groups with *falciparum* malaria in the malaria endemic counties of Busia, Siaya and Homabay in western Kenya.

Donation of Blood Sample for Laboratory Research
Consent to Participate

Introduction
You are being invited to participate in this blood sample donation study. This study is being conducted by Ms. Shillah Simiyu, a Masters student at the University of Nairobi, Institute of Tropical and Infectious Diseases (UNITID) in partial fulfillment of the Master of Science degree in Tropical and Infectious Diseases.

Study Purpose
The aim of the study is to assess the association between the ABO blood groups and *falciparum* malaria among febrile patients who will seek medical attention at either of the county referral hospitals in the counties of Busia, Siaya and Homabay in October 2015. Approximately 246 participants will be recruited into the study.

Procedure
If you agree to participate in the study, you will first consult with a medical or clinical officer who upon suspicion of malaria will refer you to the laboratory for your blood sample to be taken. You will be seated and blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. This will take about five minutes.

Risks
While on the study, you are at risk for developing a bruise around the site on your arm around the site where the needle is inserted to collect the blood sample. This is rare when blood is drawn by trained personnel. If such a bruise develops, it will resolve without treatment within a few weeks and is rarely painful. There is also a risk of excessive bleeding if you suffer from a blood clotting disorder or are taking medication that inhibits blood clotting. If either of these conditions applies to you, it would be best if you not donate blood for this study. For the reasons stated above the person who draws your blood will observe you closely during the blood draw and, if you have any worrisome symptoms or symptoms that the investigator or his associates have described to you, notify the investigator immediately.

Benefits
There is no benefit to you. The blood will be used only for laboratory research.

Voluntary participation and right to withdraw from study
Donation of blood for research is voluntary and you should not be placed under any pressure to

do so. You do not have to agree to give a blood sample nor need to explain why you should choose not to donate. There will be no consequences if you refuse to participate in the study.

Confidentiality
Any personal information provided by you in connection with the donation will be held in confidence. Information which identifies you will be kept secure and restricted. However, your personal information may be given out if required by law. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information which identifies you will be destroyed when this research is complete.

Ethical Approval
To ensure that the study conforms to research ethics, it has been reviewed and approved by the Kenyatta National Hospital-University of Nairobi Ethical Review Committee. If you have any complains about the study please contact the committee chairperson, Prof.AnastaciaGuantai on 020 2726300 or make an appointment to see her at the University of Nairobi School of Pharmacy.

Contacts:
If you need to contact the investigator on any matter relating to the study please call 0701 489004 or email shillahsimiyu@students.uonbi.ac.ke

Declaration
I have read the above information and had the opportunity to ask questions to my satisfaction. I voluntarily consent to participate in the study.

APPENDIX 3: Statement of Consent/Assent

University of Nairobi Institute of Tropical and Infectious Diseases
Investigator: Ms. Shillah Simiyu
Supervisors: Prof.Dr. Walter Mwanda
STUDY TITLE: Association of the ABO blood groups with *falciparum* malaria in the malaria endemic counties of Busia, Siaya and Homabay in western Kenya

STATEMENT OF CONSENT:
If you agree to participate in this study by filling in a questionnaire please sign below

I, , have read or have had read to me, the consent form for the above

study and have discussed the study with

I understand that the following (check the box only if you fully understand and agree with each statement):

☐ The goal of this research is to determine the association between ABO blood groups and *falciparum* malaria among febrile patients who will have sought medical attention at the county referral hospitals in Busia, Siaya and Homabay in October 2015.

☐ Participation is completely voluntary and I can withdraw from the study at any time. I am aware and give permission that the the information I give shall be analysed and disseminated but my personal identification details shall not be recorded in any analysis or report in this study.

Name of Study Participant_____
Signature: _____ Date: _____

For Research Staff:
I, _____, have explained the nature and purpose of the above study to _____
Name of Research Staff:_____
Signature: _____Date:____ _____

All study participants will be issued with a copy of this information and consent form

APPENDIX 4: Informed consent/assent - Swahili version (Kiambatisho cha tatu: Habari na fomu ya Idhini).

Chuo Kikuu cha Nairobi Taasisi yaTropikali na magonjwa ya kuambukiza (UNITID)
Mpelelezi: Shillah Simiyu
Msimamizi: Prof.Dr. Walter Mwanda.

KICHWA CHA UTAFITI: Uhusiano kati ya makundi ya damu ya ABO na *falciparum* malaria katika kaunti za Busia, Siaya na Homabay zilizo magharibi mwa Kenya.

Kutoa Kwa Hiari Kwa Damu Ili Ichunguzwe Katika Maabara
Ruhusa ya Kushiriki
Utangulizi

Unaalikwa uhusike katika zoezi hili la utafiti wautoajidamu .Utafiti huu unafanywa na Ms. Shillah Simiyu, mwanaafunzi wa shahada ya Masters, katika Chuo kikuu cha Nairobi, Taasisi ya Tropikali na magonjwa ya kuambukiza (UNITID).

Madhumuni ya Utafiti

Madhumuni ya utafiti huu ni kubaini uhusiano uliopo kati ya makundi ya damu ya ABO na viini vya malaria miongoni mwa wagonjwa wanaotafuta matibabu katika hospitali za kauntiza Busia, Siaya na Homabay mwezi Octoba 2015.Zoezi hili linakusudia kuhusisha watu 246.

Utaratibu

Iwapo utakubali kushiriki, kwanza kabisa utamuona muuguzi ambaye akishuku kwamba anaugua malaria, atakuelekeza katika maabara ambako damu yako itafanyiwa uchunguzi.Utaratibu wakutoa damu ili kufanya uchunguzi huchukua dakika tano hivi. Utaketishwa chini kisha, kwa sindano, damu itatolewa kutoka kwa mshipa mkononi.

Athari

Utakapo husika katika utafiti huu,utachibuka kidogo katika sehemu yamkono patakapo tolewa damu kwa sindano. Hata hivyo hii ni nadra sana haswa ifanywapo na mtaalamu. Jeraha linalotokea, isitoshe, litapotea baada ya siku chache na halina uchungu wala halitatizi. Pana pia hatari ya kuvuja damu kwa wingi iwapo una ukosefu wa chembechembe za damu zinazo sababisha damu kua chakuvuja au unatumia dawa zinazozuia damu kuganda kwa haraka na kuziba jeraha. Kwahivyo, ni bora kumuarifu daktari mapema iwapo una yoyote kati ya hayo ili usihusike katik autafiti huu.

Manufaa

Zoezi hili halina faida au manufaa yoyote kwako wewe binafsi. Damu utakayo toa itatumika tu kwa utafiti ndani ya maabara.

Kujihusisha kwa hiari na haki ya kujiondoa

Kushuhulika katika zoezi hili ni hiari ya mtu binafsi na hustahili kushurutishwa kwa njia yoyote ile. Hapana adhabu yoyote ukikataa kuhusika.

Hakikisho la Usiri

Habari yoyote kuhusiana na utoaji wa damu yako katika hili zoezi itabaki siri. Hata hivyo habari nyengine yoyote kukuhusu inaweza kutolewa iwapo inahitajika kisheria. Matokeo ya utafiti huu yatakapochapishwa au kujadiliwa katika mikutano au makongamano, majina yako au chochote kitakachokutambulisha hakitatumika.Habari yoyote kukuhusu itafutwa mara tu zoezi hili litakapokamilika.

Kibali cha Maadili

Kuhakikisha kuwa utafiti umejilainisha na maadili ya utafiti, uchunguzi umefanywa na utafiti ukapitishwa na kamati ya kuchunguza maadili yautafiti ya hospitali kuu ya Kenyatta -Chuo Kikuu cha Nairobi (KNH/UON-ERC). Ukiwa na malalamishi yoyote kuhusu utafiti huu tafadhali wasiliana na mwenye kiti wa kamati, Prof. Anastacia Guantai -020 2726300 au kufanya uteuzi kumwona katika Chuo Kikuu cha Nairobi.

Mawasiliano

Iwapo unahitaji kuwasiliana na anayehusika na uchunguzi katika zoezi hili tafadhali piga simu: Ms Shillah Simiyu-0701489004 au tuma barua pepe kwa s**hillahsimiyu@students.uonbi.ac.ke**

Azimio

Nimesoma ujumbe uliotangulia na nimeridhika. Nimepeana ridhaa ya kushiriki katika utafiti kwa hiari yangu.

Sahihi:

Mimi …….. nimesoma/ nimeielewa hii fomu na maswali yangu yamejibiwa. Nakubali kushiriki katika utafiti huu.

Sahihi ya mshiriki _____

Sahihi ya shahidi (kama hawezi kusoma na kuandika)_____

APPENDIX 5: Standard Operating Procedure (SOP) for Giemsa Staining Technique

PURPOSE: To provide guidelines for the proper detection, identification and quantification of malaria parasites in Giemsa stained MBFs (malaria blood films) at the county referral hospital laboratories.

APPLICABILITY: For all techs, Lab I/C and students within county referral hospital laboratories.

PRINCIPLE: Malaria blood films are stained with Giemsa stain solution which is composed of eosin and methylene blue (Azure).The eosin component stains the nucleus of the parasite which then appears red; the methylene blue component stains the cytoplasm of the parasite which then appears blue.

ABBREVIATIONS AND TERMS

- MBFs - Malaria Blood films
- IA W - In accordance with
- Q.C. - Quality control
- SOP - Standard Operating Procedures
- I/C - In-charge
- HPF-High Power Field

EQUIPMENT AND MATERIALS

- Latex gloves
- Biohazard container
- Slide-box

REAGENTS AND STAINS

- Giemsa powder
- Buffer (PH7.0-7.2)
- Giemsa stain solution
- Measuring cylinder (1000ML)
- Conical flask
- Glycerol
- Water bath
- Stirrer
- Methanol
- Glass Rod

RESPONSIBLITIES

- The Laboratory I/C and Quality control officer will ensure that this procedure is strictly adhered to and that all personnel that are required to perform this procedure are properly trained.
- TheI/C and Heads of Department will ensure the day to day adherence to this standard.
- Quality Assurance: SOPs are to be reviewed/approved by the lab I/C, office of quality, or designee, prior to the responsible approving authority.

PROCEDURES

1. Technique of preparation of Giemsa stock solution

- Weigh 3.8g of Giemsa powder and transfer into a conical flask
- Measure 250mls methanol and add to the stain mix well using a stirrer (glass rod) Measure 250mls glycerol and add to the stain. Mix well using glass rod
- Place the bottle of stain in a water bath at 50 - 60°c for 30 minutes or if not available at 37°c for up to 2 hours, to help the stain dissolve.
- Label and mark "Inflammable" indicate date and time prepared and technologist/technician initials, store at room temperature in the dark.

2. Technique of preparation of 10% Giemsa working solution.

- Pour 90 MLs of buffered water (pH 70-7.2) into a l00ml. Graduated cylinder
- Using a serological pipette or syringe. Draw up 10ml of Giemsa stain. Add the stain to the buffered water in the graduate cylinder.
- Cover the top of graduated cylinder. Gently invert the cylinder several times until completely mixed.
- Label the cylinder with contents, date prepared, and time prepared, expiration time and technician/technologist initials.
- Buffered Giemsa stain must be discarded and prepared a fresh after 7 hours

3. Technique of staining with 10% Giemsa stain solutions.

- Place the slide, on a flat staining rack.
- Pipette the stain onto each slide until the smear are completely covered or place the slide, in the staining jars then pour the stain into the jar.
- Let it stand for 10 minutes.
- Rinse the slide with gentle jet of tap water or dip three times gently in a beaker of distilled water. Replace rinsing water frequently.
- Allow the slides to dry in a vertical position

Caution: A malaria blood film that is too pink suggests low pH or over staining while that which is too blue or purple suggests high pH or under staining.

4. Examination of smears.

- Thick and thin blood films should initially be reviewed at low power(100 x magnification) particularly at the edges of the thick and thin film where microfilaria, Malaria parasites and trypanosomes may be concentrated.
- Thick film should then be examined systematically beginning in the center of the film and moving in a defined fashion out from the center. At least 200 oil immersion fields should be reviewed

(magnification x1000).

 Thin films should be examined systematically back and forth across the feathered end of the film for at least 300 oil immersion fields (magnification x 1000).

5. Method of Determining Parasitemia in thick blood films

 Count parasites and leukocytes separately

 If after 200 leukocytes have been counted, 10or more parasites have been identified, record the results in the record form indicating the number of parasites seen per 200 leukocytes.

 If after 200 leukocytes have been counted nine or less parasites have been counted, continue counting until 500 leukocytes have been counted and record the parasites observed per 500 leukocytes counted.

 Report the parasite count in parasites per microliter in relationship to the leukocyte count by the following formula; the parasites per microliter is equal to:

$$\frac{\text{\# of parasites X white blood cell count per } \mu l}{\text{\# of leukocytes counted.}}$$

6. Quantification of parasites in thick film

The following method will be used for quantifying a sexual malaria parasites forms (in either single or mixed species infections) as well as sexual (gametocytes) forms. If different species are observed, this fact will also be recorded.

 10 parasites per 100 HPF…….. +

 11- 100 Parasites per 100 HPF……..++

 10 Parasites per 100 HPF……..+++

 Over 10 parasites per HPF……. ++++

EXPECTED STAINING RESULTS

1. Thin films :

 The background should be clean and free of debris; the color of the erythrocytes is a pale grayish pink.

 Stippling should show up Schuffner's dots in erythrocytes containing *Plasmodium vivax* or *P. ovale* and Mauer's spots in erythrocytescontaining the larger ring forms of *Plasmodium falciparum*.

2. Thickfilm

 The background should be clean andfree ofdebris with pale mottled graycolor derived from the lysed erythrocytes.

 Leukocyte nuclei are a deep purple

 Malaria parasites are well defined with deep red chromatin and a pale purplish blue cytoplasm.

 In P. vivax and P. ovale infections the presence of Schuffner stippling in the ghost of the host erythrocyte can be seen.

REPORTING

 The presence of malaria parasites, the species identified and the level of parasitemia should be reported

- High levels of parasitemia(>1% or >50,000 parasites/µl)) are critical and should be reported immediately.

NOTES:

- Drain excess stain/water by tapping on the container or absorbent material to avoid liquid transfer.
- Do not fix thick film with methanol as this will inhibit lysis of RBCs to expose parasites.
- Staining this will strictly be adhered to avoid over staining or under-staining.
- Stain should always be covered when not in use to prevent evaporation/contamination.
- Avoid the use of heat on the smears before and after staining. This interferes with to colour reactions and morphology of parasites

LIMITATIONS

- It may take several thick and thin blood smears to exclude the diagnosis of malaria, particularly in semi-immune individuals or on individuals on chemo-suppressive therapy.
- The sensitivity of the thick smear is estimated to be 10-100 parasites/µl and therefore low parasitemia may be missed.
- It may be difficult to determine the species identification in cases with low numbers of circulating ring forms and in cases of mixed infections.
- If blood samples are old or if patients have received partial therapy the morphology of the parasites may be altered making species identification difficult.

REFERENCES

- Bench aids for diagnosis of malaria infections (WHO)
- Clinical diagnosis and management by laboratory methods 19th Edition (John Bernard Henry, 2001)
- National Guidelines for laboratory Diagnosis of Malaria in Kenya (Division of malaria control, 2007)

APPENDIX 6: Standard Operating Procedure for ABO blood grouping

PURPOSE: To describe the steps used in the procedure for ABO blood grouping

SCOPE: This procedure applies to the county referral hospital laboratories' blood bank section where procedure for ABO blood grouping is performed.

PRINCIPLE AND BACKGROUND: The procedures are based on direct agglutination (clumping of red blood cells carrying a specific antigen in the presence of a corresponding specific antibody

ABBREVIATIONS

- TECH- Technologist/Technician
- SOP- Standard operation procedure
- LAB- Laboratory
- IAW- In Accordance With
- QC- Quality control

EQUIPMENT

- Stop watch

MATERIALS, REAGENTS AND SUPPLIES

- Slide/Tile
- Lancets
- Applicator stick
- Clean test tubes
- Grouping anti-sera
- Normal saline
- Positive control (ideally AB cells)
- Negative control (ideally O cells)
- Latex Gloves
- Biohazard container

RESPONSIBILITY

- Only certified competent laboratory personnel may independently carry out this procedure.
- The blood bank Head of Section shall ensure that all personnel performing this procedure are sufficiently trained so as to fully perform and implement this procedure.

SAFETY

- Always follow minimum laboratory universal safety precautions.

PROCEDURE

Reagent storage and stability

- Store at 2-8◦c prolonged storage outside this temperature range may accelerate loss of reagent activity. Do not freeze.

Specimen collection and preparation

- Capillary blood or blood collected in EDTA tubes can be used.
- Test should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimen may be stored at 2˚c-8˚c for up to 24 hours.
- Bring specimens to room temperature before testing.

TEST PROCEDURE Slide technique

- Place one volume of reagent on a clear labelled glass slide. Add an equal volume of test red cells suspension.
- Mix cells with Anti -A or Anti -B reagent over an area of about 20mm diameter by gently and continuously rocking the slide, mix Anti – A, B reagents with cells over of about 20x40mm with a clear application stick, then slowly tilt the slide back and forth for 30 seconds with occasional further
mixing.
- Read macroscopically for agglutination after 2 minutes.
- Read any apparently negative test with Anti – A and Anti- B microscopically after 5 minutes.
- Tests giving weak reactions should be repeated by the tube technique.

Tube Technique

- Prepare a suspension of test red blood cells in physiological saline. Use a 3-5% cell suspension for testing with Anti – A or Anti – B.
- To one volume of reagent in labelled test tube add an equal volume of test red cell suspension.
- Mix and incubate at room temperature for one minute.
- Centrifuge at 1000 rpm for 20 seconds.
- Gently agitate the tube to dislodge the red cells and examine macroscopically and microscopically for agglutination.

READING AND INTEPRETATION

- **Agglutination:** Positive results, indicating the presence of the appropriate test red cells.
- **No agglutination**: Negative result, indicating the absence of the appropriate antigen on the test red cells.

PROCEDURE NOTES

- Prolonged storage outside this temperature range (2-8ºC) may accelerate loss of reagent activity. Do not freeze.
- False positive or false negative results may occur through contamination or test materials,improper storage or any deviation from the recommended technique.

REFERENCES

- Biotech Laboratories inserts.
- The production standardisation of monoclonal antibodies as AB group reagents (Muller and Salmon, 1983).

Appendix 7: Study Questionnaire:

First Name:…….

Surname: …….

Age: …….

Gender: …….

Village: ……..

Sub-County: ……..

County:……

Symptoms…….

Clinical Diagnosis: ……..

Have you used any antimalarial drugs within the last two weeks (Please tick): Yes ☐ No ☐

RESULTS OF LABORATORY DIAGNOSIS

ABO/Rh blood group (Please tick): A+ve ☐ B+ve ☐ AB+ve ☐ O+ve ☐

A-ve ☐ B-ve ☐ AB-ve ☐ O-ve ☐

Malaria test results: Positive ☐ Negative ☐

Malaria Parasite results (if positive): + ☐ ++ ☐ +++ ☐ ++++ ☐