ASSOCIATION OF HUMAN ABO AND RH(D) BLOOD GROUP SYSTEMS WITH MALARIA PARASITE INFECTIONS IN MIESSO DISTRICT, WEST HARARGE ZONE, OROMIA REGIONAL STATE

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Association of Human ABO And Rh (D) Blood Group Systems with Malaria Parasite Infections in Miesso District, West Hararge Zone, Oromia Regional State

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As Thesis research advisors, we hereby certify that we have read and evaluated this Thesis entitled **''Association of Human ABO and Rh(D) Blood Group Systems with Malaria Parasite Infections in Miesso District, West Hararge Zone, Oromia Regional State''** prepared under my guidance by **Cuba Gaddisa Alemu**. I recommend that it be submitted as fulfilling the Thesis requirement.

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DEDICATION

I dedicate this Thesis to all my families, relatives and to my high school teachers.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work and that all sources of materials used for the thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an MSc degree at Haramaya University and is deposited at the University library to be made available to borrowers under the rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department, Department of Biology or the Director of The Postgraduate Program Directorate when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

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BIOGRAPHICAL SKETCH

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ABBREVATIONS AND ACRONYMS

ACIPH	Addis Continental Instute of Public Health
DNA	Deoxyribonucleic Acid
CCM	Community-based Case Management
FMOH	Federal Ministry of Health
IMNCI	Integrated Management of Neonatal and Childhood Illness
IRS	Indoor Residual Spray
ITBN	Insecticide Treated Bed Net
LLIN	Long Lasting Insecticidal Net
MIS	Malaria Indicator Survey
MLDM	Manual for the Laboratory Diagnosis of Malaria
MOH	Ministry of Health
NGOs	Non-governmental organizations
OMIM	Online Mendelian Inheritance in Man
PFEMP1	*P. Falciparum* Erythrocyte Membrane Protein 1
RBCs	Red Blood Cells
RBM	Roll Back Malaria Partnership
RDTs	Rapid Diagnostic Tests
Rh	Rhesus Factor
SPSS	Statistical Package for Social Science
WHO	World Health Organization

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ABSTRACT

The ABO blood group system is a genetic polymorphism that leads to phenotypic polymorphism. The objective of this study was to evaluate the relationship between malaria, particularly P. falciparum and P.vivax malaria, and the distribution of the ABO and Rh (D) blood group systems in Miesso district of West Hararge Zone of Oromia Regional State. A case-control study design was carried out from November to January 2016. A total of 168 people participated in this study. Blood group data and socio-demographic data were collected from both groups whereas clinical data related to malaria were collected only from cases. ABO and Rh (D) blood were determined using serological method for both cases and controls, while blood films examinations for malaria parasite determination in cases. From the selected cases of malaria, 85.7% P. falciparum, 8.9% P. vivax and 5.4% were mixed case. From the total of 56 blood samples examined in cases, there were 58.9% severe malaria category and 41% mild malaria category. The frequencies of ABO blood types in the case group were A = 37.50%, B = 32.14%, O = 21.43%, AB = 8.93% and for control group A = 17.0%, B = 16.1%, O =58.9%, AB = 8.0%. Most of the cases and controls were Rh^{+}. The allelic frequencies of ABO blood group for case and control were 0.567, 0.268, 0.165 and 0.741, 0.132, 0.127 I^{O}, I^{A}, I^{B} respectively. In the current finding there were significant association between malaria parasite and ABO blood group with χ^2 = 12.605, P-value= 0.012. There were an association between ABO blood group and malaria severity level with likelihood-ratio chi^2 (6) =30.8359 and fisher's exact = 0.000 and no association between Rh blood group and malaria severity level with χ^2 = 2.643, P-value = 0.274. Generally blood types A were more susceptible to severe malaria as compared to patients with other blood types and blood types O were less susceptible.

Key words: *ABO blood group, association, malaria, P.falciparum, Rh blood group*

1. INTRODUCTION

The ABO blood group system is a genetic polymorphism that leads to phenotypic polymorphism and it is one of the classical molecular markers that are well studied since its discovery around the beginning of the 20th century (1900) (OMIM 110300). Individual humans can be categorized into one of four known ABO blood group phenotypes (A, B, AB, and O) due to possession of different surface antigens on red blood cells (RBCs) which result in difference in reaction during serological tests. These differences are due to variation in antigens on the surfaces of RBCs that are in turn due to genetic differences in a gene known as *H*, that was mapped to chromosome 9 and that encodes L-fucosyltransferase, the enzyme that adds the antigens to the surfaces of RBCs (Ahmed *et al.,* 2007).

The Rh system is the second most significant blood-group system in human-blood transfusions. The D antigen is the most significant Rh antigen which usually provokes an immune system response. Its proper blood group matching is particularly important in the prevention of Rh haemolytic disease which is a common occurrence in feto-maternal blood transfusion between a D-positive fetus and D-negative mother (Basu *et al.,* 2011; Bennardello and Curciarello, 2013; Varghese *et al.,* 2013).

Malaria is a disease caused by blood parasites of the genus *Plasmodium*. There are four types of *Plasmodium* species that infect humans, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae.* A new malaria parasite species named *P. knowlesi* is identified in Asia affecting both humans and animals (Sabbatani *et al.,* 2010**)**. Malaria parasite is transmitted from an infected person to another by the bite of a female *anopheline* mosquito (MLDM, 2012). Among the four known malaria causing protozoan species *P. falciparum* is the most devastating in its cause of morbidity and mortality. In Sub-Saharan Africa it is mainly transmitted to humans with a bite from a mosquito vector species known as *Anopheles gambiae.* Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths (WHO, 2016). Some 13 countries – mainly in sub-Saharan Africa – account for 76% of malaria cases and 75% deaths globally (WHO, 2016). In areas

with high transmission of malaria, children under 5 are particularly susceptible to infection, illness and death. More than two thirds (70%) of all malaria deaths occur in this age group (WHO, 2016). Around 2.4 billion people were estimated to be at risk of *P. falciparum* transmission in 2007 (Guerra *et al*., 2008). Africa a region of the major disease burden; where 70% of clinical events are thought to occur, with the majority of deaths among children (Snow *et al*., 2005).

Even though Ethiopia is described as relatively low malaria prevalence country compared to most other malaria-endemic countries in Africa; malaria is still the major public health problem. The disease occurs in more than three-quarters of the landmass of the country and an estimated 68% of the total populations are considered at risk of malaria infections; of which around 60% of cases are due to *P. falciparum* while the rest 40% is due to *P. vivax* in Ethiopia (ACIPH, 2009). Despite its still great burden, control of malaria is not yet succeeding. Generally, areas below 2,000 meters above sea level in altitude are considered as malaria-endemic with increased burden among children less than 10 years and *P. falciparum* causing more than 2/3 of the cases and the rest with *P. vivax* (MIS, 2012).

Some studies found association between susceptibility to *P. falciparum* and ABO blood groups system. The association was suggested four decades ago (Athreya and Coriell, 1967) and a number of studies since then have established this association, particularly with the severe form of *P. falciparum* (Cserti & Dzik, 2007; Panda *et al.*, 2011). The studies found out that individuals with blood type O are less susceptible to the severe form of *P.falciparum* malaria compared to individuals of the other blood types. The mechanism of resistance conferred by O-blood type is reported to be through reduced rosetting, the spontaneous binding of infected erythrocytes to uninfected erythrocytes, and is thought to contribute to the pathogenesis of severe malaria by obstructing micro-vascular blood flow (Rowe *et al*., 2007).

Based on different observations it was hypothesized that the evolution of blood group system has been shaped greatly by malaria parasites and vice versa. The various observations supporting this hypothesis include the association between the O-blood type and less susceptibility to *P. falciparum* malaria; the increased frequency of the O-type in malaria

endemic areas than in other areas, as well as DNA sequence analyses of different *Plasmodium* species and of humans and other primates (Cserti & Dzik, 2007). According to this hypothesis *P. falciparum* malaria has been a very important selective pressure on the distribution of the ABO blood groups in humans and in human evolution in general over the past several million years (Cserti & Dzik, 2007). In other studies conducted in India on association of ABO blood groups and malaria infections of variable severity provides evidence that 'A' blood group is more susceptible to malaria infection, and risk of cerebral malaria and disseminated intravascular coagulation (DIC) is more in 'A' group (Gupte *et al.,* 2012).

The relationship of *P. falciparum* malaria with the ABO and Rh blood groups has been given little attention in Ethiopia hitherto. Some studies includes Tekeste and Petros (2010) and Zerihun *et al*. (2011) found that severity of *falciparum* malaria is reduced in blood type O patients as compared to other blood groups studied in Ziway, Metehara and Awash. Studies done in Felegeselam Health Center, northwestren Ethiopia show that significantly higher proportions of individuals with blood groups A, B and AB have severe *P. falciparum* infection than blood type O. They also reported that there was a frequency difference between ABO blood groups of controls, ABO blood groups of uncomplicated malaria cases and ABO blood groups of severe malaria cases. Individuals with severe malaria had significantly higher parasite count than patients with uncomplicated malaria. Significantly higher proportion of individuals with blood types A, B and AB were found to have severe *P. falciparum* infection than blood type O (Hailu and Kebede, 2013).

Other studies done at Dore Bafeno and Arba Minch in Southern Ethiopia indicated considerable prevalence of malaria parasites in apparently healthy blood donors attending Arba Minch Blood Bank. Donors with blood group O are significantly more susceptible to asymptomatic malaria as compared to non-group-O donors (Getaneh and Mohammedaman, 2015).

No data on the relationship between malaria and ABO and Rh (D) blood group systems have so far been generated in the present study area. This study was done to know if there are significant differences between the different blood types (A, B, AB, and O) as well as the Rh (D) blood types in frequency of *P.falciparum* and *P.vivax* malaria infections in Miesso district and to know if there are frequency differences among the different blood types between cases of severe malaria and mild forms.

In addition to advancing our scientific understanding of the co-evolution of the pathogenesis of malaria and the human ABO and Rh (D) blood group systems, this study also has some practical significance. The study will generate data on the association of malaria and blood groups [ABO and Rh (D)] in the selected district. These data may help partly in the planning of malaria control and prevention for the districts' health departments, both governmental and NGOs (Non-governmental organization). The blood group data may also help in planning blood donation campaigns. People with susceptible blood type may be warned to take necessary precautions when moving to malaria endemic areas.

General Objective

The general objective of this study was to evaluate the relationship between malaria (both *P. falciparum* and *P.vivax* malaria) and the distribution of the ABO and Rh (D) blood group systems in Miesso district of West Hararge Zone of Oromia Regional State.

The specific objectives of this study were:

- To determine phenotypic frequencies of the ABO and Rh (D) blood group systems in study area.
- To determine allelic and genotypic frequencies of the ABO and Rh (D) blood group systems in study area.
- To test the association between the blood group systems and malaria (both *falciparum* and *vivax)* and with severity levels as well in a case-control analysis.

2. LITERATURE REVIEW

2.1. The Genetics of the ABO and Rh Blood Group Systems

Based on serological tests individual humans can be categorized into four ABO blood phenotypes: A, B, AB, and O and the ABO blood group is the most medically important blood types that very useful in assuring safe human-blood transfusions: due to antigenic differences on the surfaces of RBCs. The phenotypic variation that are caused by genetic differences in a gene known as *H*, that was mapped to chromosome 9 and that encodes L-fucosyltransferase, the enzyme that adds the antigens to the surface of RBCs (Ahmed *et al*., 2007 and Storry and Olsson, 2009). The antigens that determine ABO blood groups are oligosaccharide constituents of cell surface glycolipids and glycoprotein. These sugars are added to an existing chain of oligosaccharides (precursor molecule containing R chain to which Nacetylglucoseamine and D-galactose attached) which protrudes from the erythrocyte (RBC) membrane. The H antigen is produced by the addition of L-fucose to the terminal galactose of this precursor by an enzyme, L-fucosyltransferase (Ahmed, *et al*., 2007).

The O group has only this H antigen. In people of blood group A, *N*-acetyl-D-galactosamine (A determining) is added to the terminal D-galactose of the H antigen with the help of *N*-acetylgalactosaminyl transferase enzyme which is encoded by the *IA* allele of the *ABO* gene located on chromosome 9 (9q34.2). A variant of this enzyme known as the D-galactosyl transferase, is encoded by the *B* allele of the *ABO* locus adds D-galactose to the terminal galactose of the H antigen, to form the B antigen. Both the A and the B antigens are found on the surfaces of the RBCs of individuals with AB blood group since they are heteozygotes for the two alleles. The *O* allele does not produce a functional enzyme at this locus and there is no any addition to the H antigen. The *A* and *B* alleles are codominant while the *O* allele is recessive to both of them. The genotypes that determine the different phenotypes in the ABO system are shown in *Table 1* (Ahmed *et al*., 2007).

Table 1.The ABO and Rh blood groups, their genotypes, and antibodies produced in plasma

Genotype	Blood group	Description	Antibodies in plasma
AA	A	The gene encoding the 'A' glycosyl transferase is present on both copies	Anti-B
AO	A	The gene encoding the 'A' glycosyl transferase is present only on a single copy.	Anti-B
BB	B	The gene encoding the 'B' glycosyl transferase is present on each copy	Anti-A
BO	B	The gene encoding the 'B' glycosyl transferase is present on only a single copy	Anti-A
AB	AB	One copy has the gene encoding the 'A' enzyme while the other has the gene encoding the 'B' enzyme	neither anti-A nor anti-B
OO	O	Neither the 'A' gene nor the 'B' gene is present on either copies of the gene	anti-A and anti-B
Rh^+	Rh^+	Has a *RHD* gene encoding D-antigen	Anti –D
Rh^-	Rh^-	Has a *RHD* gene encoding D-antigen	No anti –D

The *ABO* locus contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most *O* alleles and results in a loss of enzymatic activity. The *A* and *B* alleles differ from each other by seven nucleotide substitutions, four of which translate into different amino acids in the gene product (Arg176Gly, Gly235Ser, Leu266Met, and Gly268Ala). The residues at positions 266 and 268 determine the A or B specificity of the glycosyltransferase they encode (Yamamoto *et al*., 1990). The O allele differs from the A allele by deletion of guanine at position 261. The deletion causes a frameshift and results in translation of an almost entirely different protein that lacks enzymatic activity. The *A* allele is sometimes subcategorized into two variant forms (*A1* and *A2*) (Yamamoto *et al*., 1990).

The Rh system is the second most significant blood-group system in human-blood transfusions. The D antigen is the most significant Rh antigen which usually provokes an immune system response. Its proper blood group matching is particularly important in the prevention of Rh haemolytic disease which is a common occurrence in feto-maternal blood transfusion between a D-positive fetus and D-negative mother. The Rhesus factor is clinically the most important protein-based blood group system With 49 antigens; it is the largest of all 29 blood group systems. The unusually large number of Rhesus antigens is attributable to its complex genetic basis. The antigens are located on two Rhesus proteins - RhD and RhCE - and are produced by differences in their protein sequences. (Basu *et al.*, 2011; Bennardello and Curciarello, 2013, Varghese *et al.*, 2013).

The Rh locus is located on the long arm of chromosome 1 (on 1p36-p34). It contains the *RHD* and *RHCE* genes, which lie in tandem. The RHD and RHCE genes are structural homologs and result from a duplication of a common gene ancestor. RHD and RHCE each contain 10 exons and span a ~75-kb DNA sequence. The RHD gene is flanked by two 9-kb, highly homologous sequences called "Rhesus boxes". It is thought that unequal homologous recombination confined to the Rhesus boxes is a common cause of the deletion of the RHD gene, which is found in up to 40% of the population (Wagner, 2005).

The *RHD* gene arose from the duplication of an ancestral *RH* gene during mammalian evolution. An *RHD* deletion occurred during the evolution of hominids, so that many modern humans completely lack the *RHD* gene. This haplotype is the leading cause of the D negative phenotype worldwide. The *RH* alleles can be grouped according to their molecular structure. For the most part, these groups' show point mutations (SNP, single nucleotide polymorphisms) which cause missense, nonsense, frame shift or splice site mutations. *RHD-CE-D* hybrid alleles are often formed by gene conversion. The gene codes for the RhD protein on the red cell membrane. D^- individuals who lack a functional RHD gene do not produce the D antigen, and may be immunized by D^+ blood as shown in table 1. The *RHD* gene was found two years later, and the total deletion of this gene ascertained as the cause of the European D negative phenotype (Dtsch, 2007).

2.2 .The ABO Blood Group Antigens

The finding of Adam *et al.,* 2007 shows that host genetic factors modulate the risk and severity of malaria infection via specific mediators, which can be different in various epidemiological settings. RBC invasion by *P.falciparum* merozoites and the adhesion of parasitized RBCs to other cells play a central role to *falciparum* malaria path physiology. 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). The antigens of the ABO blood group system are oligosaccharides 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 locus in RBCs is in the forms of —Lipid—Glucose—Galactose—N-acetyl glucosamine— Galactose—Fucose as indicated on figure 1. The A allele of the ABO locus codes for N-acetylgalactosaminyl (GalNAc) transferase which catalyzes the formation of an α-1, 3 glycosidic bond between the outermost galactose component of the O antigen and GalNAc (figure 1). 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 (figure 1). 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.

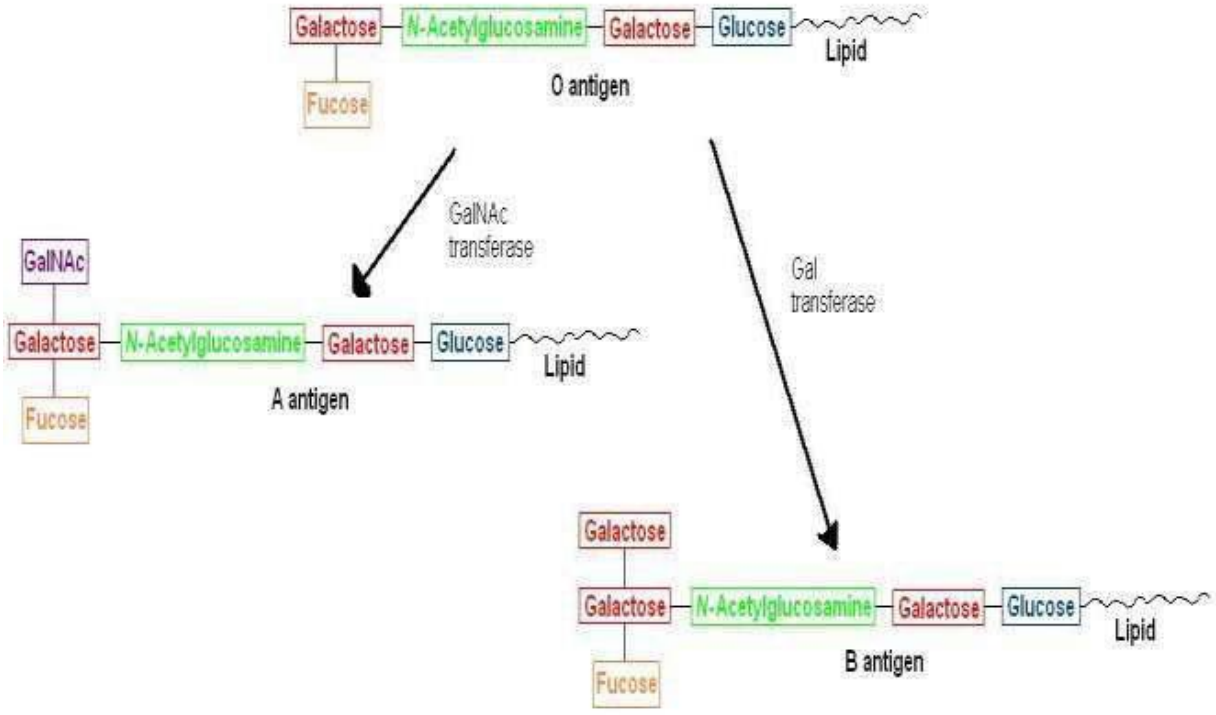

Figure 1. Structures of A, B, and O Oligosaccharide Antigens (Shillah Nasambu Simiyu, 2015).

Abbreviations: Gal, galactose; GalNAc, N-acetyl galactosamine.

2.3. The Hardy-Weinberg Genetic Equilibrium

The Hardy-Weinberg "equilibrium" refers to this stability of allele frequencies over time (James, 1999). The principle of Hardy-weinberg genetic equilibrium provides the solution to how variation is maintained in a population with Mendelian inheritance. According to this principle, the frequencies of alleles (variations in a gene) will remain constant in the absence of selection, mutation, migration and genetic drift. The genotype frequencies can be predicted from the allele frequencies. For example, in the simplest case of a single locus with two alleles: the dominant allele is denoted A and the recessive allele a and their frequencies are denoted by *p* and *q*; frequency (A) = p; frequency (a) = q; p + q = 1. If the genotype frequencies are in Hardy-Weinberg proportions resulting from random mating,

then we will have frequency (AA) = $p2$ for the AA homozygote in the population, frequency of $q2$ for the aa homozygote, and frequency $2pq$ for the Aa heterozygote (Russell, 2005).

p2 (AA): 2pq (A a): q2 (aa)

An important application of the Hardy-Weinberg law is estimating the genotype frequencies in a population. The majority of the deleterious recessive genes in human population are carried in heterozygous condition. To calculate the frequency of individuals with different genotypes we usually begin by counting the number of homozygous recessive individuals; these homozygous individuals can be distinguished from the rest of the population by the phenotype. By using the Hardy-Weinberg law we can calculate the frequency of the heterozygous and homozygous conditions (Cummings, 2000). For this study, the frequencies of the ABO blood group genotypes and alleles were calculated or estimated using the extension of the Hardy-Weinberg law as employed by (Griffith *et al.,* 2008). In other words, when you add up the frequency of the A, B and O alleles, you have accounted for 100% of the alleles for this gene that are present in the population .The genotypic frequencies are given by the following equation, when the allelic frequencies are p = A, q = B and r = O.

(p + q + r) 2 = p2 (AA) + 2pq (AB) + q2 (BB) + 2pr (AO) + 2qr (BO) + r2 (OO) (Griffith *et al.,* 2008).

Three alleles are computed (A, B and O), with frequencies equal to p, q and r respectively. The frequencies of the genotype at equilibrium will be computed by the square of the allelic frequencies. This system has six possible genotypic combinations but only four phenotypic blood groups. Because the alleles A and B are co-dominant and both are dominant to O Homozygous AA individuals and heterozygous AO individuals are phenotypically identical, as are BB and BO individuals. This results in four phenotypic combinations, known as blood types A, B, AB, and O.

2.4. Life Cycle of Malaria Parasites and Pathogenesis

The life cycle of *Plasmodium* involves humans and mosquitoes (female *Anophles* mosquitoes). The *Plasmodium* species exhibits three life-cycle stages in the human host as shown in figure 2. Those are the sporozoites, merozoites and the gametocytes stage. The sporozoite stage of the parasite is transmitted to the human host via the bite of an infected mosquito; some of which are able to invade the liver cells for development into schizont stage which then bursts to release thousands of merozote stage into blood after about a week. This stage invades RBCs and multiplies to produce more merozoites within 2-4 days producing fever and vital organ damage. After several generations, some merozoites develop into sexually differentiated forms known as the gametocytes (male and female forms) in infected RBCs. Both male and female gametocytes are taken up by the mosquito when feeding on human blood, gametes fuse in the mosquito gut, and the zygote (ookinete) penetrates the gut wall to form an oocyst; which, after 10 to 14 days, releases thousands of sporozoites which then move to the salivary glands for another cycle of human infection. The most severe clinical manifestations of malaria are seen with *P. falciparum* where cerebral malaria, severe malarial anaemia, and respiratory distress may result, in some cases with fatal results (Ahmed *et al*., 2007; MLDM, 2012).

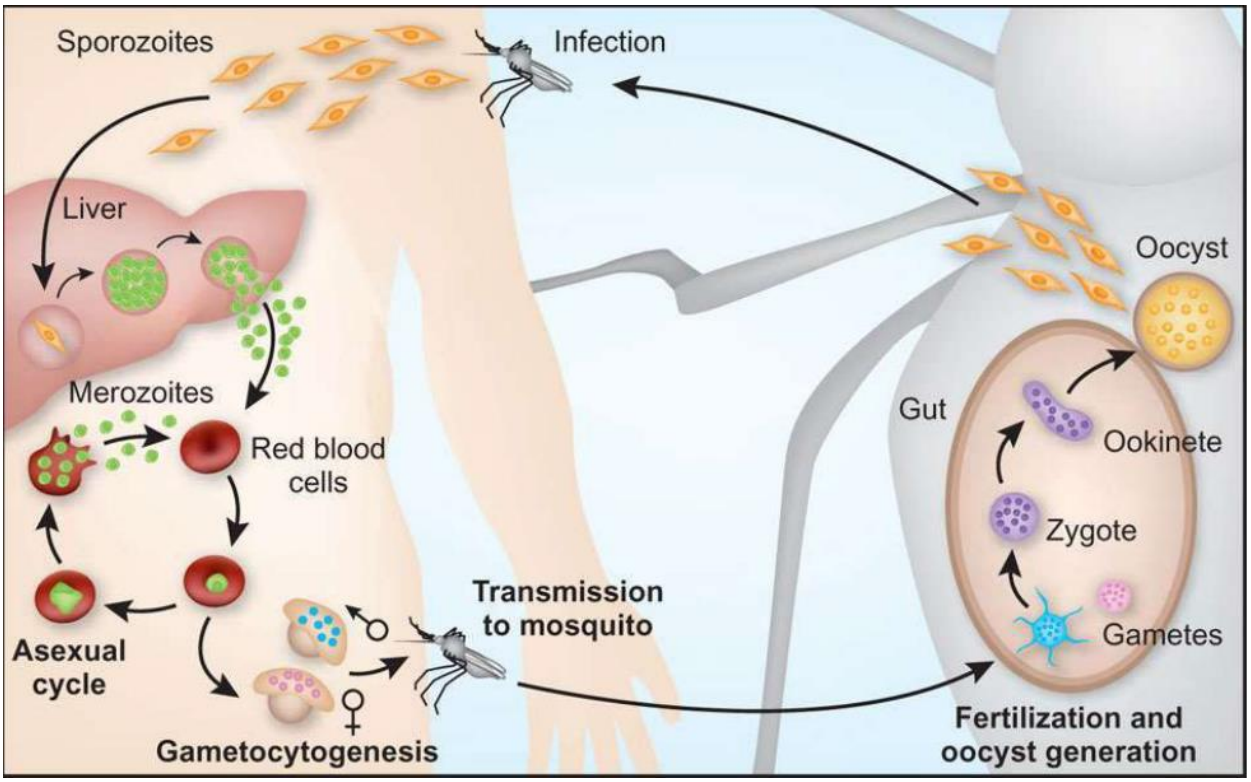

Figure 2: Life cycle of *Plasmodium falciparum* (Pasvol, 2010)

The pathogenesis of human *P falciparum* infection is a complex interplay of parasite-induced RBC alterations and microcirculatory abnormalities accompanied by local and systemic immune reactions, resulting in multiple clinical forms of variable severity (Grau *et al*., 2003, Maier *et al*., 2009, 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. 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-7 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 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), Eselectin, chondroitin sulphate A (CSA) and P-selectin (Newbold *et al*., 1999) 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).

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 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 parasetimia.

2.5. Epidemiology and Geographic Distribution of Malaria

2.5.1. Global Epidemiology and Geographic Distribution of Malaria

Killeen *et al*., (2002) reported that the overwhelming bulk of the world's malaria burden rests upon the population of sub Saharan Africa because of the remarkable expanding of human populations, weak health systems, the world's most efficient vector mosquito species and environmental conditions ideal for transmission. Malaria occurs throughout most of the tropical regions of the world. *P. falciparum* predominates in Africa, New Guinea, and Haiti. *P. vivax* is more common in Central America. The prevalence of these two species is approximately equal in South America, the Indian subcontinent, eastern Asia, and Oceania. *P. malariae* is found in most endemic areas, especially throughout sub-Saharan Africa, but is much less common. *P. ovale* is relatively unusual outside of Africa and, where it is found comprises < 1% of isolates (Nicholas *et al*., 2008).

Evidently anopheles has co adapted to human ecosystem in the Afro tropical savannah where their combined contribution to malaria transmission has apparently facilitated evolution of falciparum malaria (Mukabana *et al*, 2006). An exploratory survey of malaria prevalence and people's knowledge, attitudes and practices of mosquito larval source management for malaria control in western Kenya shows malaria prevalence of 3.2–6.5% in all 8 sites studied. Nevertheless, residents perceived malaria as their major health risk. Thirty-two percent (29/90) of all respondents did not know that mosquitoes are responsible for the transmission of malaria. Over two-thirds (69/90) of the respondents said that mosquito breeding site could be found close to their homes but correct knowledge of habitat characteristics was poor (Imbahale *et al*., 2010). A study in Kipsamoite, Nandi District, Kenya an epidemic-prone rural highland with unstable malaria transmission showed that, the most frequent initial sources of treatment for malaria in adults and children were medical facilities (66.0% and 66.7%) and local shops (19.0% and 30.3%) (Sumba *et al*., 2008).

2.5.2 Epidemiology and Geographical Distribution of Malaria in Ethiopia

According to FMOH (2005) and Shargie *et al*., (2008) malaria is one of the country's foremost health problem, top ranking in the list of common communicable diseases and in 2004/05, it was reported as the leading infectious disease followed by helminthiasis and tuberculosis. As the result shows, more than 65% of the 70 million people has been reported to be exposed to the disease. Nevertheless, due to low service utilization rate, the potential health service coverage is accessible to about 61% of the population. About 52 million people in Ethiopia faced the risk of malaria (FMOH, 2008).

The finding of Tilahun *et al., (*2009) reported that the epidemiological pattern of malaria transmission in Ethiopia is generally seasonal and highly unstable due to variations in topography and rainfall patterns. Marked variations in the level of transmission from place to place or seasonal fluctuations in the number of cases are the main features of malaria transmission in Ethiopia. According to FMOH, (2005) the major transmission of malaria follows the June to September rains and occurs between Septembers to December while the minor transmission season occurs between April to May followed by the February to March

rain. As a result of the short peak transmission and the relatively long duration of low transmission during the dry season, people are highly vulnerable to malaria due to lack of acquired immunity that comes with frequent exposure to malaria infections, resulting in the occurrence of frequent epidemics (Tilahun *et al*., 2009.

As reported by Kassahun (2004) three-fourths of the land below 2000 masl is malarious with two thirds of the country's population at risk. The Dega zone of Ethiopia (altitude above 2,500 meters) with a mean annual temperature of 10- 15 degree Celsius is malaria-free. Much of the Woina Dega zone (Altitude 1500–2500 masl) is also malaria free, especially the zone in the 2000–2500 meters above sea level. Malaria in Ethiopia often occurs below 2000 meters, with short-lived transmission following the rains. However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector (Gebreyesus *et al*., 2006).

2.6. The Malaria Vector *Anopheline* Mosquitoes And Their Geographical Distribution

There are about 400 species of *Anopheles* mosquitoes in the world. Many species can transmit malaria, and of these only 60 are of major importance as vectors. Some *Anophelines* prefer to bite animals and thus either does not normally transmit malaria parasites to humans or do so very. Some others do not live long enough for the parasite to develop in the mosquito, or the parasite does not seem to be able to develop (WHO, 2002). Among many species of *Anopheles* fauna found in Ethiopia some are:

1. *Anopheles arabiensis* (*Anopheles gambiae*)

The major malaria vector in Ethiopia is *Anopheles arabiensis*. It is widely distributed in the country and is usually the vector of epidemic malaria. *Anopheles arabiensis* mainly breeds in small, temporary, and sun-lit water collections such as rain pools. It becomes abundant at the beginning and end of the big rainfalls. However, it can also breed in a wide variety of other types of water bodies. It is usually an indoor-resting species, but with an exophagic

feeding habit (WHO, 2002). It is found in all parts of Ethiopia both in lowland as well as in the highland areas up to 2000 m above sea level (Ashenafi, 2008).

2. *Anopheles pharoensis*

It is the second most frequent and widely distributed vector species of malaria in Ethiopia. It breeds in large, permanent and shaded water bodies with emergent vegetation. Lake shores and irrigation canals are also favorable breeding places. It is found along river *Baro* and *Awash,* in Lake *Tana* and in the *rift valley* lake regions. Its indoor and outdoor feeding and resting habits are generally similar to that of *A. arabiensis* (MOH and WHO, 2002).

3. *Anopheles funestus*

It is the third most common vector of malaria. It dominates in areas where malaria is endemic especially along rivers, around lakes and shaded swamps in the lowlands where altitude is between 1000m and 1500m. This vector is found along river *Baro,* Lake *Tana* and the lake regions of *Shoa* and *Sidamo* (MOH, 2002).

4. *Anopheles nili*

It is the least common and more localized species and is not adequately studied except in *Gambella*. It is found in the south western, western and north western parts of Ethiopia along river *Bilate,* the *Segan* river valley and the *Baro* River (Yibro, 2014).

2.7. Overview of Methods for Malaria Diagnosis

Prompt and accurate diagnosis of malaria is part of effective disease management. The diagnosis of malaria is based on clinical suspicion and on the detection of parasites in the blood (parasitological or confirmatory diagnosis). High sensitivity of diagnosis in malaria endemic areas is particularly important for the most vulnerable population groups, such as young children and the non-immune population, in whom the disease can be rapidly fatal, while high specificity will reduce unnecessary treatment with anti-malarial drugs and improve diagnosis of other febrile illnesses in all settings. Thus, high quality malaria diagnosis is important in all settings (MLDM, 2012).

2.7.1 Clinical Diagnosis of Malaria

A clinical diagnosis entails making a clinical assessment by taking an accurate history of the illness and performing a physical examination. Clinical diagnosis of malaria is made in a patient who has fever or history of fever in the last 48 hours and lives in malaria-endemic areas or has a history of travel within the last 30 days to malaria-endemic areas. Basing the diagnosis on clinical features alone is not recommended, as this often has low specificity and increases the chances of the patient being misdiagnosed. Unless there is an ongoing malaria epidemic, or is a peak malaria transmission season, careful laboratory testing typically reveals confirmed malaria parasites in fewer than half of clinically suspected malaria in most situations in Ethiopia. Malaria treatment based on clinical diagnosis must be the last option when there is no availability of RDTs or microscopy (MLDM, 2012).

WHO recommends universal parasitological diagnosis of malaria to ensure targeted use of antimalarial drugs for those who actually have malaria. The health worker examining a suspected malaria case should perform differential diagnosis to look for other causes of fever (e.g., typhoid fever, relapsing fever, acute respiratory tract infections, meningitis, etc) and manage the case accordingly. Malaria should still be considered, even if the individual has another obvious cause for the fever. The national algorithm of the Integrated Management of Neonatal and Childhood Illness (IMNCI) and Community-based Case Management (CCM) should also be employed for the management of the sick child presenting with fever (WHO, 2008)

The clinical course of malaria infection may be uncomplicated or severe. Because of its frequent and severe complications, *P.falciparum* is the most serious malaria-causing parasite and cause of death. Patients under the age of five, pregnant women, non-immune individuals of all ages and people living with HIV are particularly at risk for severe and complicated malaria and death (MLDM, 2012).

2.7.2 Laboratory Diagnosis of Malaria

Once malaria is suspected on clinical grounds, it is mandatory to obtain the laboratory confirmation of the presence of malaria parasites. Clinicians could request for diagnostic

test for malaria to confirm the diagnosis of malaria in a patient with symptoms and signs suggestive of malaria disease; to rule out malaria infection in a patient with other known causes of fever; to confirm malaria in febrile infants under 3 months of age; to look for treatment failure; and to investigate causes of anaemia, jaundice or splenomegaly (MLDM, 2012).

2.7.2.1 Common diagnostic methods

The two laboratory diagnostic methods or tools most often used for confirming a diagnosis of malaria are:

A. Rapid Diagnostic Tests – RDTs: RDTs detect antigens (proteins produced by malaria parasite) in the blood of a patient with malaria. **B. Light Microscopy**: Good quality microscopy is the most acceptable method for detecting and identifying malaria parasites from the blood of a suspected patient. The procedure consists of collecting a finger-prick blood sample; preparing a thin and thick blood films; staining the films with Giemsa or other stains such as Field stain and examining the film through a microscope for the presence of malaria parasites (MLDM, 2012).

2.8. Malaria Prevention and Control Methods

2.8.1. Treatment and isolation of infected people

Antimalarial drugs, including Quinine, Chloroquine and Mepacrine can be used either to prevent infection or against established parasites. Although parasites can be eliminated from the blood, the disease is difficult to cure because a reservoir of infection often remains in liver cells. Isolation using mosquito nets or similar devices is effective in preventing the parasite from passing to new vectors but is not practicable in areas where a large proportion of the population is affected. Insecticide-treated materials are important in malaria control because when used widely in the community, they have been shown to reduce transmission of the disease. The evidence from several studies show that use of insecticide-treated materials reduced infectious malaria cases in children by about 45% and all cause mortality by about 20% (MOH, 2002).

Another key intervention to control malaria is the use of insecticide treated bed net (ITBN). It has been shown that ITBNs can have a major impact on malaria burden, decreasing both morbidity and mortality (Lengler, 2004). Furthermore, indoor residual spraying (IRS), the use of long-acting chemical insecticides on the walls and roofs of houses in order to kill and repel the mosquitoes, has been proven to be an effective way to reduce malaria transmission, especially in areas with low and variable/seasonal transmission (Mabaso *et al.*, 2004).

2.8.2. Drainage of Breeding Grounds

The larval stage of mosquito requires static or stagnant water to develop. Filling ponds and draining marshes close to human habitation reduces the number of adult mosquitoes able to transmit the disease (Ashenafi, 2008).

2.8.3. Spraying Houses with Insecticide

This is the most effective method of control as to Roll Back Malaria reported (2003), the walls of houses are sprayed with a persistent insecticide to kill adult mosquitoes. Houses should not be built near to pools, streams or water holes. No holes should be made near a house. Barrels and tanks to collect rain water must be so made the mosquitoes cannot enter. In the house persons should sleep under a mosquito net. Europeans may wear special tall mosquito boots to protect themselves from being bitten. A house may be sprayed with pyrethrum, which kills mosquitoes.

Indoor spraying is one of the most valuable tools in malaria vector control. It was the strategy used in the most successful eradication programmers of the 50's and 60’s (RBM, 2003). As the name implies, IRS involves coating of the walls and other surfaces of houses with a residual insecticide. For several months, the insecticide will kill mosquitoes and other insects that come in contact with these surfaces (CDC, 2008). In addition to the above, biological control of mosquito vectors involve the introduction of natural enemies into mosquito breeding sites. These could be in form of parasites or predatory animals like fish, insects, fungi, nematodes etc (WHO, 2002).

2.8.4. Eradication of Larva

In large parts of the world where malaria was occurring, the anti-malaria program was aimed at controlling the *Anopheles* mosquito rather than eradicating it. It was done by killing the mosquito's larva in their aquatic stage; it is called larva control. In addition to this, it was done by insecticide spraying in the air of bedroom and cleaning drainage facilities around settlements. However, controlling methods were economically feasible only in towns and other settlement areas with marked economic values like mining and industrial areas, and in military barracks and camps. Malaria has mainly been a rural disease, which has affected large numbers of population residing in developing countries of Latin America, Asia and Africa. The program was extremely difficult and it couldn't achieve its goal (Mabaso, 2004).

Muller demonstrated for the first time in 2001 how DDT was a powerful insecticide to kill mosquitoes. He showed that it killed insects without being ingested, but only being touched by the insects' limbs. It could be lethal to insects for several months after it is sprayed on a surface. It does not cause any serious harm to human beings and other domestic animals, unless it is ingested by them (Mabaso, 2004). *Anopheles* mosquitoes rest on the wall of houses after sucking blood from human beings. It was believed that it would be effective to use DDT or other residual insecticides like chlordane, dieldrin, hexachlorocyclohexane (HCH), on the wall of the houses. In doing so, the mosquitoes could easily absorb the insecticide within their body and die immediately (WHO, 2002). There are several useful chemotherapeutic antimalarial agents recommended by World Health Organization. To date Chloroquine is the preferred primary antimalarial treatment recommended by the WHO for malaria areas where Chloroquine-resistant malaria is not common. For Chloroquine-resistant *Plasmodium* situations WHO recommends artemisinin-based combination therapy (ACT) (WHO, 2002).

The two main major vector control activities implemented in Ethiopia are IRS and LLINs. The 2007 MIS showed significant improvements in LLIN ownership in malaria risk areas

from 3.5% in 2005 to 65.6% in 2007 (MIS, 2007). It appears the more than 20 million LLINs that have been distributed to 10 million families have contributed to the reduction of malaria, and the strategies and activities required to implement this have now been tried and tested. The objective of this component is to ensure that 100% of households in malarious areas own one LLIN per sleeping space, and that at least 80% of people at risk of malaria use LLINs. This will be achieved by both covering the existing gap (catch-up) and replacing worn out nets (keep-up), geographically targeting households in need. IRS is currently targeted to cover epidemic-prone areas and malaria-affected communities with low access to the health care system. Despite a dramatic scale-up of IRS activities, the FMOH estimates that 55% of IRS-targeted areas have been sprayed. This Strategic Plan aims at increasing and maintaining IRS coverage to 90% of households in IRS-targeted areas. Geo-coding activities will help determine the quantity, quality, and location accessibility of human habitations, as well as measure sprayable surfaces within a specific area (Draft National Strategic Plan for Malaria, 2010).

As the nation, the regions, and sub-regions seek to reduce malaria transmission to zero, the very high coverage (seeking universal coverage) of prevention interventions (LLINs, IRS) will serve to limit transmission in communities and will mean that there is very little potential for “epidemics” or “outbreaks”; that is, there is very low likelihood that a single introduced infection would expand to many infections/cases. Thus, the national emphasis on further reducing transmission will rely on the existing prevention coverage and additionally focus on “surveillance”– the process of finding of individual human cases, treating and performing case investigation to identify the source and possible spread, with the aim of preventing any further malaria transmission. Therefore, this NSP aims to achieve a high quality, broadly based malaria infection detection, investigation and response ‘Surveillance System’ to further reduce malaria transmission and improve the detection and timely response to malaria epidemics (Draft National Strategic Plan for Malaria, 2010).

2.9. The Role of Malaria on the Distribution of the ABO Blood Types and Mechanism of Variation in Pathogenesis

Selective pressure from infectious diseases of human are believed to have played a greater role in shaping human evolution to produce the current pattern of human population genetic diversity. Malaria is particularly a very important selective factor of human genetic variation. A large group of human genes (more than 30) have already been implicated in susceptibility to malaria many of which are associated with *P. falciparum* (López *et al*., 2010). It was hypothesized that, a long time ago, *P. falciparum* malaria had significant influence on the evolution and diversity of ABO blood group antigens (Athreya and Coriell, 1967). This hypothesis has not been supported with enough data until recently.

Support for the hypothesis that *P. falciparum* malaria had significant influence on the evolution and diversity of *ABO* blood group antigens came from the analyses of a broad range of available data that suggested that the origin, distribution, and relative proportion of the *ABO* blood groups in humans may have been directly influenced by selective pressure from *P. falciparum* infection (Cserti & Dzik, 2007). They put forward four arguments in support of this hypothesis:

First, because deletion 261 is found in all populations worldwide, it presumably arose during evolution in Africa before the outward migrations of early humans. DNA sequence information dates the emergence and development of the *O* allele from *A* to a period of evolution (around 5 million years ago) before human migration out of Africa (originated 200,000 years ago and migrated out of Africa around 100,000-40,000 years ago) consistent with the effect of *P. falciparum* (diverged from chimpanzee's *P. reichenowi* 9-10 million years ago; and current genetic form originated 50,000-27,000 years ago). The most intense malarial selection pressures were effectively applied to the human genome in the relatively recent period (10,000-3000 years ago) when agricultural development favored *Anopheles* mosquito and this was consistent with the observed development of multiple adaptive erythrocyte mutations.

Second, the current global geographic distribution of group O is also consistent with a selection pressure by *P. falciparum* in favor of group O individuals in malaria-endemic regions. Accordingly, Group O is generally higher than others in tropical and subtropical regions where *P. falciparum* is (was) endemic. In contrast, group A is the predominant blood group in the colder regions of the Earth, where malaria has not been endemic.

Third, there is a correlation between disease severity and *ABO* group where group O individuals are less affected with severe malaria compared to other groups. Association with malaria has been suggested first before four decades ago (Athreya and Coriell, 1967) and a number of studies since then have established this association, particularly with the severe form of *P. falciparum* including in Ethiopia (Cserti & Dzik, 2007; Panda *et al*., 2011; Tekeste & Petros, 2010; Zerihun *et al*., 2011 and Getaneh and Mohammedaman, 2015). The studies found out that individuals with blood group O are less susceptible to the severe form of *P. falciparum* malaria compared to individuals of the other blood groups. Fourth, analysis of available data on pathogenesis of *P. falciparum* infection led to the proposal of a biologic model that summarizes the role of ABO blood groups in cytoadherence biology. The mechanism of resistance conferred by O-blood group is reported to be through reduced rosetting (Alexandra *et al*., 2007).

2.10. Association between ABO Blood Group System and 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 parasitemia. 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).

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 there by having 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), there by confirming the hypothesis that group A blood group correlates with disease severity. In Ethiopia, Teketse *et al*. (2010) assessed 210 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%). 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.

According to Shillah (2015) malaria infection showed significant association with A and AB blood group and the highest proportion of infection 44% was observed among respondents with blood group A followed by those with blood group O 39.4%. Blood group O was not statistically associated with getting malaria infection. The results of this study on the relationship between ABO blood groups and malaria showed that children with blood group A experienced malaria attacks more frequently than those in the other blood groups but the observed differences in the prevalence of malaria infection among the children of the various blood groups were not significant ($P > 0.05$). This therefore suggests that there is no significant association between ABO blood groups and malarial infection.

3. MATERIALS AND METHODS

Details of the materials and methods including participants, the data collected, and methods used to analyze the data are presented in this section.

3.1. Description of the Study Area

Miesso is one of the districts in West Hararge Zone of the Oromia Regional state, eastern Ethiopia as shown on figure 3 below. Miesso town is the capital of the district, and the geographical location of the area ranges from 8° 48″ 12′ – 9° 19″ 52′ latitude 40° 30 9 and 40° 56″ 44′ E longitudes and altitude varying from 1107 to 3106 meter above sea level according to Tamiru *et al*., 2014. It is predominantly categorized under hot and warm sub-moist agro-ecological zone receiving annual average rain fall 727 mm distributed in bimodal pattern. The first rainy season extends from March to may while the second (main rainy season) from June to September. The annual mean minimum and maximum temperature of the district is 15 and 30.6^{o}C respectively (MoA, 1998 and NMSA, 1996 in Tamiru *et al*,. 2014).

It is found at a distance of 238 km from Harar town and 287 from Addis Ababa, the capital of Ethiopia, on the main road connecting the two cities. It is, the lowland district of West Hararge, is on the plain at base of the mountains of mount Asebot. Asebot is one of the towns of Miesso district and it is under malaria endemic region. It is known that generally areas in Ethiopia with altitudes below 2000 masl are expected to be malaria endemic areas. It is one of the districts labeled to be malaria endemic regions (personal communication from Oromia Health Bureau malaria expert). The map of the study area was presented on the following figure (figure 3).

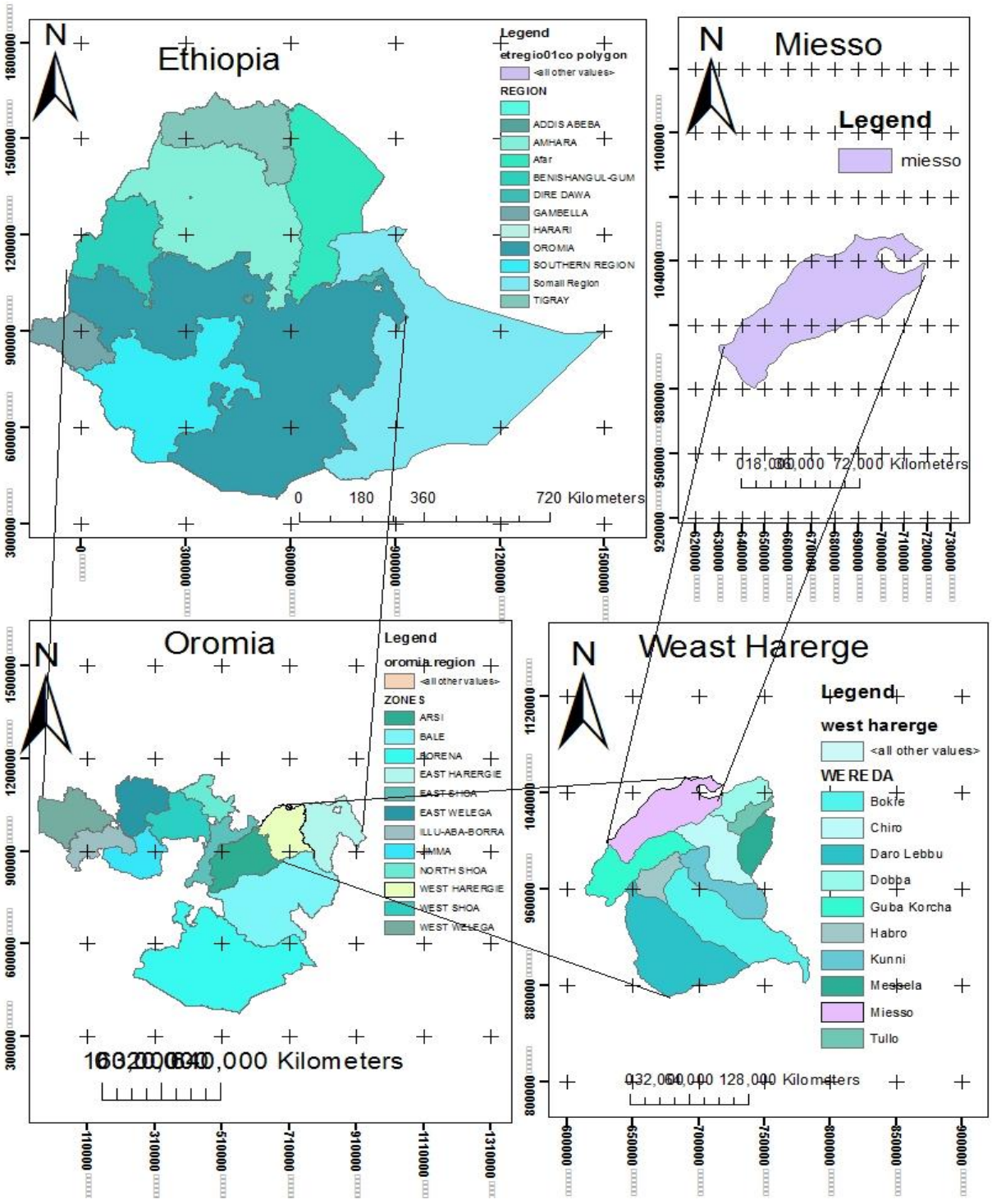

Figure 3. Map of Study Area

Source: - GIS / Geographical Information System

3.2. Study Design

To undertake association analysis between *P. falciparum*, *P. vivax* malaria and mixed status (cases with varying degrees of severity and controls) and the blood groups (A, B, AB, and O in ABO and Rh^+ and Rh^- in Rh-factor) a case-control study design was used. A case-control study is designed to help determine if an exposure is associated with an outcome (i.e., disease or condition of interest). In theory, the case control study can be described simply. First, identify the cases (a group known to have the outcome) and the controls (a group known to be free of the outcome). Then, look back in time to learn which subjects in each group had the exposure(s), comparing the frequency of the exposure in the case group to the control group. By definition, a case-control study is always retrospective because it starts with an outcome then traces back to investigate exposures. When the subjects are enrolled in their respective groups, the outcome of each subject is already known by the investigator.

Controls should be chosen who are similar in many ways to the cases. The factors chosen to define how controls are to be similar to the cases are the matching criteria. The selected control group must be at similar risk of developing the outcome. For the current study relatively the case and control matched by sex and the source of case and control were the same in residence and time of hospitalisation. The study was conducted in Miesso district of West Hararge zone of Oromia Regional State during one of the two pick malaria transmission seasons; from November 2016 to January 2016.

3.3. Background and Source of Populations

The source population for the study was permanent residents of Miesso district who were supposed to use the district's health facilities (Asebot Health Center and Miesso *woreda* Health Center) for treatment.

3.4. Study Population

The study population was febrile patients who visited the two health centers who were malaria positive for the cases and for the control group non febrile patient of the district who are assumed to be from the same background population as the cases.

3.5. Inclusion and Exclusion Criteria

3.5.1. Inclusion Criteria

A permanent resident of the district, who were a member of a dominant (in terms of population size) ethnic group (self declared) in the district, and who give a signed consent to participate in the study were included in the study. All malaria cases who visit Asebot health center and Miesso *woreda* Health Center were included in the study. As control group an individual free from malaria at the time of test period were selected as a member of control group from the same population as case participant.

3.5.2. Exclusion Criteria

Individuals who did not fulfill the above criteria (Section 3.5.1) were excluded from the study.

3.6. Sample Size Determination

The sample size included in the study were much depended on the number of malaria positive cases in the two health centers (Miesso and Asebot) during the study period: November to January 2016, who were selected purposively after giving consent to participate. A total of 56 cases were obtained and the number of the control group was determined as twice of the number of cases therefore a total of 112 malaria free controls were included in the present study; making the total sample size of 168 participants.

3.7. Sampling Procedure/Technique

All clinical cases of malaria confirmed using thick and thin blood film who visit Asebot health centers and Miesso woreda health centers were asked to voluntarily participate in the study. Criteria such as unrousable coma, repeated seizures were used to distinguish severe cases of *falciparum* malaria of any age from the mild (uncomplicated) cases. The malaria parasite positive cases that had clinical symptoms such as fever, chills, shivering, headache, loss of appetite and rarely joint pains (arthralgia) and generalized muscle ache (myalgia) were classified into uncomplicated (mild) malaria. Control groups were sampled from

healthy residents (malaria free at the time of the test period) of the district who are assumed to be from the same background population as the cases. Persons that were relatives of the patients that accompany the patients to the health facilities were included in the control group.

3.8. Data Collection Methods

All the clinical data including the blood groups were collected following standard procedures by nurses and laboratory technicians. In addition, some simple sociological and personal data were collected through questionnaire in the form of interview method by researcher (Appendix II).

3.8.1. Blood Sample Collection and Blood Group Determination

The blood samples were collected from each sample individual after their agreement to participate in the research process and signed the consent forms that assured their willingness. The blood sample was collected totally from 168 study participants (n = 56 cases and n = 112). The blood collection was performed by using lancet, to obtain a drop of blood from each individual finger. Blood samples were taken from finger pricks, and open slide method of testing for ABO blood groups and Rh (D) factor was followed. A three drop of blood from each sample individual was taken and divided in to three clean slides. (Slide A, B, and D). A drop of each of the anti sera(anti sera A was added to slide A, anti sera B was added to slide B and anti D was added to slide D) and mixed with each blood sample. Blood was mixed thoroughly with the anti sera and rocked gently until agglutination observed. Blood group was determined on the basis of agglutination and recorded, as blood group A+, B+, AB+, O+ or A-, B-, AB- and O-. The blood samples were collected by laboratory technician following a standard clinical procedure with lancet, slides, and chemicals like Anti- A, Anti-B and Anti –D.

3.8.2. Parasite Type Detection

In accordance with the National Malaria guidelines of 2012, malaria microscopy is the sole technique employed in hospital and health center levels (MLDM, 2012). Blood samples were collected from finger pricks of each individual case participants (n = 56 cases). Two

drop of blood from a sterilized finger from each individual were obtained and appropriate thick and thin blood film on one slide were prepared using 10% Giemsa solution at a PH of 7.2 for staining. Thin blood smear was rapidly air-dried, fixed in anhydrous methanol and stained; then the red blood cells (RBCs) in the tail of the film was examined under oil immersion (times 1000 magnification). Thin blood film was used to assist in the identification of the malaria species after the parasites have been seen in the thick film.

3.9. Socio-demographic and other Data

Some socio-demographic data of the participants such as age, grade, sex, self-declared ethnicity, residence of the study participant were collected using questionnaire in the form of interview (Appendix II).

3.10. Data Quality Control

The quality of the data collected was checked and rechecked for quality at all stages of the research; from data collections through to entering them into excel for data analyses. To avoid spurious association between blood group(s) and malaria at analysis level the controls was selected from the same background population as the cases. The clinical data was collected by trained personnel (nurses and laboratory technicians) to have reliable data.

3.11. Methods of Data Analysis

All the collected data were immediately inserted in SPSS software version 16.0. Summary statistics such as means, standard deviations was calculated for age variable. Summary statistics for distribution of ABO blood group for case and control was presented in simple graphs. Summary statistics for sociodemographic characteristics of the participant case and control, distribution of Rh blood group of cases and control, Allelic and genotypic frequency of ABO and Rh blood groups of case and control, observed versus expected ABO and Rh (D) blood group of case and control, ABO blood group respondent and parasite infection, cross tabulation of age by severity level of study participants, cross tabulation of severity level by sex and age of the case respondent and cross tabulation ABO and Rh(D) blood group and severity level case and control are presented in table from.

3.11.1. Association Analyses

Associations between blood groups, ABO (A, B, AB, and O) and Rh (D) (Rh-D Positive and Rh-D Negative) and malaria case-control status or disease severity status (severe and mild) were done using a chi-squared test SPSS. All reported P- values were two-sided and ($P \leq 0.05$) was considered as statistically significant.

3.11.2. Estimation of Allele and Genotype Frequencies

For this study three alleles were computed (I^A, I^B and I^O), with frequencies equal to p, q and r, respectively. The frequencies of the genotypes at equilibrium are computed by the trinomial expansion $(p + q + r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) (Griffith *et al.*, 2008). The four blood group phenotypes were indicated as A, B, AB and O. The frequency of the blood group phenotypes was calculated as the number of individuals belonging to the phenotypic.

Formula for the calculation of allelic frequency

$p = 1 - \sqrt{B + O}$

$q = 1 - \sqrt{A + O}$

$r = \sqrt{O}$, (p, q, r) denote allele frequencies and A, B, O blood groups

A correction factor (d) was calculated according to $d = 1 – p – q – r$. Then the final corrected allele frequencies would be:

$P' = P (1+ d/2)$

$q' = q (1+ d/2)$

$r' = (r + d/2) (1+ d/2)$

Formula for the calculation of genotypic frequency will be

$I^A I^A = P^2$ for homozygote *AA*

$I^A I^O = 2pr$ for heterozygote *AO*

$I^B I^B = q^2$ for homozygote *BB*

$I^B I^O = 2qr$ for heterozygote *BO*

$I^A I^B = 2pq$ for heterozygote *AB*

$I^O I^O = r^2$ homozygote *OO*

And the formula for the calculation of expected phenotypic frequency that is the number of Individuals belonging to different phenotypic classes.

Expected number of A = p^2 + 2pr

Expected number of B = q^2 + 2pr

Expected number of AB = 2pq

Expected number of O = r^2

Observed and expected genotype frequencies of Hardy Weinberg were calculated on the basis of genotypic frequency and Chi- square ($\chi 2$) test was done to test the independence and the goodness of fit for genotypic frequency. Allelic frequencies were calculated under the assumption of Hardy Weinberg equilibrium and expressed as percentage. Chi- square test was used to compare observed genotypic frequency distribution of the blood groups and Rh antigen to the expected Hardy-Weinberg equilibrium (Chakraborty, 2010).

$$\chi^2 = \sum \frac{(\text{Observed Number} - \text{Expected Number})^2}{\text{Expected Number}}$$

3.12. Ethical Consideration

This study followed the ethical standards and requirements set by the University. Each participant (mothers/caretakers of children under 18) was provided a written informed consent after the purpose and objectives of the study was explained by the researcher. Letter of support was obtained from Haramaya University department of biology and Mieso *Woreda* health office.

4. RESULTS AND DISCUSSION

4.1. Socio-demographic Characteristics of the Participant

The socio-demographic characteristics of the participants of the study are shown in table No. 2. A total of 168 respondents were included in the present study, of which 53.6% were females and the rest 46.4% were males. From the participants 56 were malaria cases and 112 controls assumed from the same background as cases were included. In this study more malaria cases were detected in age groups of 11-20 years. From the case participants 53.6% were previously taken medication for treatment for the current infection of malaria.

Table 2. Sociodemographic Characteristics of the study Participants of case and control

Variables		N		Percent (%)	
		Case	Control	Case	Control
Sex	Female	23	66	41.10	58.90
	Male	33	46	58.90	41.10
	Total	56	112	100.00	100.00
Age	0-10	10	4	17.90	3.60
	11-20	31	32	55.40	28.60
	21-30	13	49	23.20	43.80
	>31	2	27	3.50	24.10
	Total	56	112	100.00	100.00
Previously who taken medicine (cases)	Yes	30	-	53.60	-
	No	26	-	46.40	-
	Total	56	-	100.00	-

4.2. Frequency Distribution of ABO and Rh (D) Blood Group Systems

As the result shown in table 3 in the current study from a total of 56 cases the most frequent ABO blood type was blood type A 37.50%, the second more frequent ABO cases were blood type O 32.14%. Blood type B and AB accounts 21.43% and 8.93%, respectively. Distribution of ABO blood group in cases were in the order of A > O > B > AB. The ABO

blood type for control was in the order of O > A > B > AB with frequency of 66, 19, 18 and 9 respectively. The least frequent ABO blood type in cases and control were AB blood type. In this study most of the controls were blood group type O (58.9%) Most probably the individual with A blood type were infected with malaria as indicated on the table (Table 3).

Table 3.Distribution of ABO blood group of ABO blood group of case and control

ABO blood group	N		Percent (%)	
	Case	Control	Case	Control
A	21	19	37.50	17.0
B	12	18	21.43	16.1
O	18	66	32.14	58.9
AB	5	9	8.93	8.0

As shown in table 4 In the current study most of the case and control participant were Rh positive 89.3% and 95.5% respectively and few of cases and control were Rh negative but, Rh negative individuals were more frequent in cases than in healthy controls. Generally the Rh blood group systems of the study participant were Rh positive (Table 4).

Table 4.Distribution of Rh blood group of case and control

Rh blood group	N		Percent (%)	
	Case	Control	Case	Control
Rh^-	6	5	10.70	4.50
Rh^+	50	107	89.30	95.50
Total	56	112	100.00	100.00

As indicated on table 5 in the current study the participant with O Blood type were the most frequent blood type followed by blood type A, B and AB (Table 5). This result is relatively in line with findings of Getaneh and Mohammedaman, 2015. They reported that the most frequent ABO blood group was blood group O, which accounts 42.1%, followed by group A accounting for 32.7%, Blood groups B 0.9% and AB 4.3% of the donors,

respectively. Most of the donors, (92.8%), were Rh+, while only (7.2%) were Rh-. Also the current finding is in relatively line with Zerihun *et al.*, 2011 and Ayele *et al*., 2014. There results shows that, high percentage of O blood group phenotype and low percentage of AB blood group was observed among the study participant. The ABO phenotypes shown in Zerihun *et al.*, 2011 were 39 %, 34 %, 21 % and 6% for each of O, A, B and AB blood groups, respectively and Rh factor records: 91%, 9% for Rh+ and Rh- respectively in the population.

Table 5. Distribution of ABO blood group among study participant

ABO blood group	N	Percent (%)
A	40	23.80
B	30	17.90
O	84	50.00
AB	14	8.30
Total	168	100.00

The current result is consistent with some previous studies that also reported high frequency of group O and low frequency of group AB phenotypes in malaria endemic regions (Getaneh and Mohammedaman, 2015, Tekeste and Petros, 2010 and Zerihun *et al.*, 2011). On the other hand, other studies reported high prevalence of blood group 'A' and low prevalence of blood group O phenotypes in colder regions where malaria has not been endemic Uneke *et al.*, 2006 in Zerihun *et al.*, 2011.

4.3. Allele and Genotypic Frequency of ABO and Rh (D) blood group

In the present finding the allelic frequencies of ABO blood group of case show a high frequency of the allele I^O over I^A and I^B, but the allele frequencies of *A* in case were higher than allele frequency of *A* in control. Also in Rh blood grouping system the allele frequency were in the order of allele *D* higher than that of allele *d* in cases (Table 6). Generally in the study area the allelic frequencies of ABO blood group for both case and control were in the order of ($I^O > I^A > I^B$) for total study population.

Table 6. Allelic and genotypic frequency of ABO and Rh blood group of case

Allele	Frequency	Genotype	Frequency Genotype	Phenotype	Frequency Phenotype %
I^O	0.567	*OO*	0.3215	O	32.15
I^A	0.268	*AA*	0.0718	A	37.57
		AO	0.3039	A	
I^B	0.165	*BB*	0.0272	B	21.43
		BO	0.1871	B	
		AB	0.08844	AB	8.84
I^D	0.673	*DD*	0.4529	D(Rh+)	89.30
		Dd	0.4401	D	
I^d	0.327	*dd*	0.1069	d(Rh-)	10.69

As shown in table 7 the present finding the allelic frequencies of ABO blood group in control group were in the order of $I^O > I^A > I^B$ that were 0.741, 0.312 and 0.127 respectively. Also in Rh blood grouping system the allelic frequency of the control were in the order of a $D > d$ (Table 7).

Table 7. Allelic and Genotypic Frequency of ABO and Rh Blood Group of Control

Allele	Frequency	Genotype	Frequency Genotype	Phenotype	Frequency Phenotype %
I^O	0.741	*OO*	0.5491	O	54.91
I^A	0.132	*AA*	0.0174	A	21.30
		AO	0.1956	A	
I^B	0.127	*BB*	0.0161	B	20.43
		BO	0.1882	B	
		AB	0.0335	AB	4.83
I^D	0.789	*DD*	0.6225	D(Rh+)	93.45
		Dd	0.3329	D	
I^d	0.211	*dd*	0.0445	d(Rh-)	4.45

4.3.1. The Chi-square Test

The application of extended Hardy Weinberg principles for three or more alleles yields little variation in the observed and expected genotypic frequency and numbers. The calculated Chi-Square value for ABO blood group for case was 0.000535 these were not significant with $P > 0.05$ (Table 8).

Table 8. Observed Versus Expected ABO Blood Group of Case

Blood group	Observed Number (O)	Expected Number (E)	Difference (O-E)	d^2	d^2/E
A	21	21.041216	-0.041216	0.00169877	0.000081
B	12	12.0027	-0.0027	0.0000079	0.00000061
AB	5	4.95264	0.04736	0.00224297	0.00045288
O	18	18.003384	-0.00338	0.0000115	0.00000064
Total	56				χ^2= 0.000535 P > 0.05

In the current study the calculated Chi-Square values for ABO blood group for control were 5.42 and these were not significantly different with P > 0.05 (Table 9).

Table 9. Observed Versus Expected ABO Blood Group of Control

Blood group	Observed Number (O)	Expected Number (E)	Difference(d) (O-E)	d^2	d^2/E
A	19	23.861376	-4.8614	23.63321	0.99044
B	18	22.886416	-4.886416	23.87706	1.0432853
AB	9	3.755136	5.244864	27.508	3.05651
O	66	61.4992	4.5008	20.2572	0.329389
Total	112				χ^2 = 5.42 P > 0.05

The calculated Chi-Square value for Rh (D) blood group for both case and control were also not significantly different p-value greater than 0.05 (Table 10).

Table 10. Observed Versus Expected Rh blood group of Case

Blood group	Observed Number (O)	Expected Number (E)	Difference (O-E)	d2	d2/E
Rh+	50	50.011198	-0.011976	0.00014	0.0000028
Rh-	6	5.34645	-0.6	0.42713	0.0798899
					$\chi 2 = 0.07989$
					P > 0.05

The calculated Chi-Square values for Rh (D) blood group for case were 0.000093. These result shows that the expected and observed Rh blood group of case were not significantly different with P-value greater than 0.05 (Table 11).

Table 11. Observed Versus Expected Rh Blood Group of Control

Blood group	Observed Number (O)	Expected Number (E)	Difference (O-E)	d^2	d^2/E
Rh+	107	107.01365	-0.013648	0.0001863	0.0000017
Rh-	5	4.98635	-0.013648	0.0001863	0.0000374
					$\chi^2=0.000039$
					P > 0.05

4.4. Malaria Parasites among the Cases

The current result in table 12 shows that malaria cases of total 56 patients turned out to be positive for malaria and all the samples were evaluated by thick and thin smears. In the current study *P. falciparum, P. vivax* and mixed infection were detected. The current study shows that *Plasmodium falciparum* was the highest of the species responsible for malaria infection which accounts 85.7% and there is low infection of *Plasmodium vivax* and mixed case which accounts 8.9% and 5.4%, respectively (Table 12). This is in line with findings of Zerihun *et al*., 2011, Tadesse, 2013, Yibro, 2014 and Simon-Oke, 2016.

They found out that there was high malaria infection with *P. falciparum* than other species. This high rate of *P.falciparum* in the study area could be due to the availability of mosquito

vectors for the transmission of infection and suitable environmental conditions for the multiplication of *P. falciparum* and may be not for the other species.

Table 12. Distribution of *Plasmodium* Species among the Cases (n= 56)

Type of parasite	N	Percent (%)
P.falciparum	48	85.70
P.vivax	5	8.90
Mixed	3	5.40
Total	56	100.00

In the present finding the highest proportion of individuals in all blood groups were infected with *P.falciparum* as compared with other parasite. From all blood types A blood type and O blood type were more affected with *P.falciparum* than other blood types (Table 13).

Table 13. ABO Blood Group Respondent and Parasite Infection

Blood group	*P.falciparum* positive	*Vivax* Positive	mixed positive
A	17	3	1
B	11	1	0
AB	4	0	1
O	16	1	1
Total	48	5	3

As shown on appendix table 1 the current finding indicates that from the total of 56 blood samples examined in the case participants there were 58.9% severe malaria category and 41% mild malaria category (Appendix table 1). On average cases who were with severe malaria were 14.3 years old in which there mean age were less than individuals with uncomplicated malaria (mean age (21.2). However, there was no significant difference in mean age between the severe malaria, uncomplicated malaria and healthy controls (χ^2 = 29.350, P =0.169) (Appendix table 1). These finding is related with the finding of Tekeste and Petros (2010). They reported that Individuals suffering from most severe malaria symptoms were on average younger than individuals with uncomplicated malaria and

control and as there was no significant difference in mean age between the severe malaria, uncomplicated malaria and control group.

From total 33 severe malaria 37.5% were males which higher than that of females which account 21.4%. The current study shows that there were no statistical significance between both sex and malaria severity level (χ^2=6.951 and P-value = 0.065). This shows that there is no association between sex and malaria severity level among study participant (Appendix table 2).

4.5. Association of ABO and Rh (D) Blood Group and Malaria Parasite Infection

In the present study from a total of 56 cases there were 37.5%, 21.4%, 32.14%), 8.94% A, B, O and AB blood type respectively (Table 14). From a total of 112 control groups there were 17%, 16.1%, 58.9%, 8% were A, B, O, AB blood type respectively. The current study shows from a total 56 cases 58.9% were severe malaria category. From these severe cases 32.1%, 12.5%, 7.14%) and 7.14% were blood type A, B, O and AB patients respectively. Severe malaria category showed at least one sign and symptom of severe malaria from coma and seizures which were used specially in this study for identifying severity level (Table 14).

Table 14 indicates that among the uncomplicated malaria cases 5.4% were of blood type A, 8.9% were blood type B and 25% belonged to blood type O. There was high percentage of Blood group A patients in the severe malaria category than in either uncomplicated malaria or healthy controls and low percentage of Blood group O patients in the severe malaria category than in either uncomplicated malaria or healthy controls. Blood group O was found to be the dominant blood type in both uncomplicated malaria (25%) and healthy controls 58.9% and both B and AB blood type were susceptible to severe malaria (Table 14).

Malaria with most of the severe case individuals were blood type A than other blood type but both B and AB blood type were related with severe disease (Table 14). The current result is in line with some other studies such as 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 there by confirming the hypothesis that group A blood group correlates with disease severity.

Table 14. ABO blood group of the respondent and Severity

	Blood group of respondent (ABO)				Total
	A	B	O	AB	
Severe	18(32.1%)	7(12.5%)	4(7.14%)	4(7.14%)	33(58.9%)
Mild	3(5.4%)	5(8.9%)	14(25%)	1(1.8%)	23(41.1%)
Total	21(37.5%)	12(21.4%)	5(32.14%)	5(8.94%)	56(100%)
Controls	19(17%)	18(16.1%)	66(58.9%)	9(8%)	112(100.00%)

The current finding shows that there were significant association between ABO blood group and malaria parasite infections with χ^2 = 12.605 and P-value = 0.012 (Appendix table 3). The current finding shows there were significance association between ABO blood group of case and ABO blood group of control with Chi-Square of 17.419 and P-value of 0.048 (Appendix table 4). The current result shows there was no significant association between Rh blood group and malaria severity level (χ^2 = 2.643 and P-Value=0.274) so that P-value is greater than 0.05 there was no association of between malaria severity level and Rh blood group system (Appendix table 5).

The current finding shows that there is significant association between ABO blood group and malaria severity level with likelihood-ratio chi^2 = 30.8359 and Fisher's exact = 0.000 (Appendix table 6). The current study goes with previous reports suggesting that individuals with blood groups A, B, and AB are more susceptible to severe *P. falciparum* infection than those with O phenotype (Appendix table 6). These may be as a result that blood group O protects against severe malaria because protects itself by the mechanism of reduced rosetting and sequestration (Alexandra *et al*., 2007). These result is in agreement with those of Zerihun *et al*., (2011) and Panda *et al*., (2011) who reported that individuals with blood groups A, B and AB are more susceptible to severe malaria with blood group A being the most susceptible, while individuals with blood group O have a protective advantage.

5. SUMMARY CONCLUSION AND RECOMMENDATION

5.1. Summary

The general objective of this study was to evaluate the relationship between malaria (both *P. falciparum* and *P. vivax* malaria) and the distribution of the ABO and Rh (D) blood group systems in Miesso district of West Hararge Zone of Oromia Regional State during one of the two pick malaria transmission seasons; from November 2016 to January 2016.

In this study a case-control study design was used and case and controls were selected from the same background population. Some simple socio-demographic character and personal data were collected through questionnaire in the form of interview. Criterion such as unrousable coma, repeated seizures was used to distinguish severe cases of *falciparum* malaria from uncomplicated malaria. To determine malaria parasite from 56 cases blood samples were collected by finger-prinking. Thick and thin blood smears were prepared and examined microscopically after staining with 3% Giemsa solution. The presence of malaria parasites on thick blood smear was examined and the identification of *Plasmodium* species from the thin blood smear was done through oil immersed objective (100x).

The ABO and Rh (D) blood grouping, of cases (56) and control respondents (112) were determined using commercial kits based on serological test of agglutination. The ABO blood group of each subject were determined using cell grouping antisera A, antisera B and antisera D. The current result shows that most frequent ABO blood in study population was blood type O (50%), followed by blood type A which accounts 23.8% and the blood type B accounts 17.9%. The least frequent ABO blood type was blood type AB which accounts 14 (7.3%). Rh positive blood type was the most frequent blood type in the study area. In the study area the current study shows that *Plasmodium falciparum* was the highest of the species responsible for malaria infection 85.7%.

Most probably individuals under 20 have severe malaria. The age mean average individuals suffering from most severe malaria symptoms were on average of 14.3 years old and younger than individuals with uncomplicated malaria and healthy controls. There is no significant association between age, sex and malaria severity level. The frequencies of ABO

blood type of case were 37.50%, 32.14%, 21.43% and 8.93% for A, O, B and AB respectively and for control 58.9%, 17.0%, 16.1%, and 8.0% O, A, B and AB respectively. A blood type was the most frequent blood type in malaria cases in the current study. Severe malaria category showed at least one sign and symptom of severe malaria from coma and seizures which was used specially in this study for identifying severity level. There was high percentage of Blood type A malaria cases in the severe malaria category than in either uncomplicated malaria or healthy controls and low percentage of Blood group O patients in the severe malaria category than in either uncomplicated malaria or healthy controls.

5.2. Conclusion

The current result shows that there were most frequent O blood type phenotypes and least frequent AB Blood type phenotypes in the study population. In Rh blood group system the current study reveals that there were most frequent Rh^+ individuals in the study population. The study revealed that on the basis of the criteria used to determine severity of malaria, cases with blood type A were more susceptibility to severe malaria as compared to patients with other blood types and blood type O were less susceptibility to severe malaria as compared to patients with A, B, and AB blood types. The current study showed that there were a statically significance association between ABO blood group and severity level of malaria parasite infection. A blood type was more susceptible to severe malaria and O blood type was resistant to severe malaria. The current finding shows there is significance association between ABO blood group of case and control and no association between malaria infection and Rh blood group system. *P. falciparum* malaria shaped the distribution of ABO blood group which has significant influence on the evolution and diversity of ABO blood group antigens.

5.3. Recommendation

- Health education and promotion of community education should be given by health departments, both governmental and NGOs about malaria control and prevention for the Miesso *Woreda.*

- People with A, B, AB blood types should take necessary precautions when moving to these malaria endemic areas.
- The present study only employed coma and repeatedly seizures as sign and symptoms determine the association of ABO blood groups and severity level of malaria. The study also did not consider factors like parasitemia, haemoglobin concentration, and cerebral malaria. Further in-depth studies are required to clearly establish the association between malaria and ABO and Rh blood group system using more laboratory markers and more malaria severity level sign and symptoms.
- In the present study low sample size population are used to determine the association of ABO blood groups and severity level of malaria in the study area. So that other studies are needed by taking more sample size population.

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7. APPENDICES

7.1. Appendix- I

Appendix table 1. Cross tabulation of severity level by age of study participants

Severity	N	Mean age	Std. Deviation	χ^2	P-value
Severe	33(58.90%)	14.30	5.115		
Mild	23(41.1%)	21.20	8.752		
				29.350	0.169

Appendix table 2. Cross tabulation of severity level by sex of the case respondent

		Severity level		
		Severe	Mild	Total
Sex	Female	12(21.4%)	11(19.7%)	23(41.10%)
	Male	21(37.5%)	12(21.4 %	33(58.90%)
	Total	33 (58.90%)	23(41.10%)	56(100.00%)
χ^2 =5.494, P-value = 0 .065				

Appendix table 3.Cross tabulation of malaria with ABO blood group

		ABO blood group				Total
		A	B	O	AB	
	Control	19	18	66	9	112
	Malaria++	21	12	18	5	56
	Total	40	30	84	14	168
χ^2 = 12.605, P-value = 0.012						

Appendix table 4. Cross tabulation of AB O blood group of case and control

Group identification	ABO blood group of the respondent				Total
	A	B	O	AB	
Case	21	12	18	5	56
Control	19	18	66	9	112
Total	40	30	84	14	168

χ^2 =17.419 , P-value =0.048

Appendix table 5. Cross tabulation of Rh blood group of the respondent with severity

	Rh blood group of the respondent		Total
	Rh-	Rh+	
Severe	4	29	33
Mild	2	21	23
Control	5	107	112
Total	11	157	168

χ^2 = 2.643, P-value = 0.274

Appendix table 6. Fisher exact test for ABO blood types and malaria severity level among the participants.

Severity level	ABO blood group				Total
	A	B	O	AB	
Severe	18	7	4	4	33
Mild	3	5	14	1	23
Control	19	18	66	9	112
Total	40	30	84	14	168

Likelihood-ratio chi^2 (6) = 30.8359

Fisher's exact = 0.000

7.2 Appendix- II

CONSENT FORM

Association of Human ABO and Rh (D) Blood Group Systems with Malaria parasite Infections in Miesso District, West Hararge Zone, Oromia Regional State

HARAMAYA UNIVERSITY

The purpose of the study is **to evaluate the relationship between malaria (both *P. falciparum* and *P.vivax* malaria) and the distribution of the ABO and Rh (D) blood group systems in Miesso district of West Hararge Zone of Oromia Regional State**. Therefore, I will ask you some questions related to malaria and examine you the signs and symptoms of malaria and will take blood sample to examine for malaria parasite. Please be assured that the information will be confidential since participation is based on your willingness. However, your kindly participation would play key role in the success of this study. In addition, no personal identification will be written and we assure you that whatever information you are providing will only be used for the research purpose and the data will be handled only by the researcher. For people under 18 (for children) their guardians will be responsible. Are you willing to participate in the study?

Agreed □ Not Agreed □

Guardians:

Agreed □ Not Agreed □

Thank you

7.3. Appendix –III

QUESTIONNAIRE (ENGLISH VERSION)

Sample number: _____

Haramaya University

College of Natural and Computational Sciences

Department of Biology

MSc in Genetics Program

1. Sex:

Male □ Female □

2. Age _____

3. Residence _____ Father: _____ Mother: _____

4. Ethnic group _____ Father: _____ Mother: _____

5. Have you taken any medication for malaria treatment for the current malaria infection (for cases)?

Yes □ No □

6. Malaria clinical severity in cases (patients):

- Method(s) of diagnosis for malaria parasites: thick smear, thin smear or both

---

- Parasite Type detected: *P.falciparum* □ *P.vivax* □ mixed □
- Severity level: Severe vs. mild cases, by traits such as unrousable coma, repeated seizures and fever, chills, shivering, headache, loss of appetite, rarely joint pains and generalized muscle ache. respectively

_____

7. Blood group (ABO and Rh (D) in that order):

_____

7.3. Appendix –IV

CONSENT FORM (AFAN OROMO VERSION)

Kaayyoon qorannoo kanaa walitti dhufenyaa maxxantu busaa fi gosota dhigaa nama ABO fi Rh (D) giddu jiru aanaa Miesso godina harargee lixaa naannoo oromiyaa jiru maadaluuf fi beekuuf. Kanaafuu Gaaffilee waa'ee kanaan wal qabate, mallattolle busaa isin irratti mul'atu isin gaafachuu fi dhigaa kessan fudhachun maxxantu busaafi gosa dhigaa kessan baru waan barbaaduuf fedhii keessan irratti hundooftanii akka irratti hirmatan kabajaadhaan isin gaafadha. Hirmaannaan isin qorannaa kana keessatti gootan milkaa'ina qorannoo kanaatiif gahee guddaa taphata. Odeeffannoon isin naaf kennitan dhimma qorannoo kanaatiif qofa olaa waan ta'ef iccitti kessan dabarafame hin kennamu.Da'imman wagga 18 gadittif maatin isaani ykn guuddiftonni itti gaffatmumma qabu.

Qorannoo kana keessatti hirmaachuudhaaf Eeyyamamaadha?

Eeyyamamaadha_____ Eeyyamamaa miti_____

Maati daa'immanittif

Eeyyamamaadha_____ Eeyyamamaa miti_____

7.5. Appendix- V

QUESTIONNAIRE (AFAN OROMO VERSION)

Lakk : _____

1. Saala

Dhiraa □ dhaala □

2. Umuri _____

3. Iddoo Jirenyaa_____ kan Abbaa: _____ kan Haadha: _____

4. Lammumma _____ kan Abbaa: _____ kan Haadha: _____

5. Qorichaa yaala busaa yeroo amma kana kessa fudhate jirta? (Dhukubsataf)

Eye □ Miti □

6. Sadarkaa dhukuba busaa dhukusata (dhukubsataf)

- Maala itti maxxantuun busaa qoratame (thin, thick, lamanu)

_____

-gosa maxxantu busaa argame: falsiparemi □ vayvax □ maka □

-Sadarka dhukkubsata: baay'ee itti hammata ykn giddugalessa ta'u beekuuf mallatowan akka hiribba hamma irra dedebi'e baay'ee dhukubu dhubsata busaa hamma qabuf fi mallatowwan akka bowwo mata, fedhi nyaata dhabu, hollanna fi qorachisa dhukkubsata dhukkuba giddugallessa qabuf gargaaramuun adda bahu.

_____

7. Gosa dhigaa (ABO fi Rh tarttiban)

_____

7.5. Appendix VI

Probability values for chi-square analysis

Table 1. Probability Values for Chi-Square Analysis

	Probabilities								
df	0.95	0.90	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	.004	.016	.15	.46	1.07	1.64	2.71	3.84	6.64
2	.10	.21	.71	1.39	2.41	3.22	4.61	5.99	9.21
3	.35	.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35
4	.71	1.06	2.20	3.36	4.88	5.99	7.78	9.49	13.28
5	1.15	1.61	3.00	4.35	6.06	7.29	9.24	11.07	15.09
6	1.64	2.20	3.83	5.35	7.23	8.56	10.65	12.59	16.81
7	2.17	2.83	4.67	6.35	8.38	9.80	12.02	14.07	18.48
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.21
	← Acceptable →								Unacceptable

Note: From Statically Tables for Biological and Medical Research (6th ed.), Table IV, by R . Fisher and F.Yates, Edinburgh: Longman Essex, 1963.

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HARAMAYA UNIVERSITY
Building the Basis for Development

DEPARTMENT OF BIOLOGY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

November 1, 2016

TO WHOM IT MAY CONCERN

This letter is in reference to Mrs. Cuba Gaddisa (SGS/0242/08) who is a Graduate student in the Department of Biology (MSc in Genetics) at Haramaya University. Having finished course works and proposal development and defense, Mrs. Cuba is now ready to embark on field data collection for her MSc thesis research entitled "**Association of Human ABO and Rh(D) Blood Group Systems with Malaria Parasite Infection in Miesso District, West Hararge Zone, Oromia Regional State.**" Now that her research proposal is approved both at the Department of Biology by Departmental Graduate Council and at the Postgraduate Program Directorate of the University, she is expected to move-onto collect required samples from Miesso district health facilities.

This is therefore, to kindly request any concerned body to provide with any possible help or assistance during her field trip(s) to the specified place to collect data and to other places for the purchase of required chemicals and materials.

I highly appreciate any help she would receive from you in advance!

With kind regards,

Tamiru Oljira (PhD)
Head, Department of Biology

Office :+251-25-5530380, Haramaya University, College of Natural and Computational Science, Department of Biology, Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia.

Lakk 2/112/xxH/-9-35

Guyyaa 25/2/2004

Bufata Faayya Miessotiif tiif

Dhimmii;Waa,ee Deegarsa Akka gotaniif isiin gaafachu ilaala.

Akkuama Armaan oliti ibsamuuf yallamet Baratuu Kubaa Gadisa Baratu Haroo Mayaa Univaersiti kan taate Biology Departmenti kan taate Reaserchi Waa, ee Malaria Iratii Risarchi Akaa hojjetu Godina Kenyatii waan Ergamaniif godininis Gara Ana kenyati Hojjachu waan barbadef jecha Baratuu isinitii Ergiree tanaaf Deegarsa baarbachisa Akka gotaniif kabajan isin gaafana .

Nagaya Wajjiin

Mohammed Yuesuf