ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES



THE ABO BLOOD GROUP AND *PLASMODIUM FALCIPARUM* MALARIA IN AWASH, METEHARA AND ZIWAY AREAS, ETHIOPIA.

By

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ABBREVATIONS AND ACRONYMS

a s l	**above sea level**
CDC	**Center for disease control and prevention**
CI	**Confidence Interval**
CM	**Cerebral Malaria**
CSA	**Central Statistics Agency**
DCs	**Dendritic cells**
Hb	**Hemoglobin**
HC	**Healthy control**
ICAM-1	**Intracellular Adhesion Molecule- 1**
NKTCs	**Natural killer T cells**
OD	**Odd Ratio**
PfEMP-1	***P. falciparum* erythrocyte membrane protein-1**
PfEMP-2	***P. falciparum* erythrocyte membrane protein-2**
PfEMP-3	***P. falciparum* erythrocyte membrane protein-3**
RDT	**Rapid malaria diagnosis test**
TSP	**Thrombospondin**
SM	**Severe malaria**
UM	**Uncomplicated malaria**
VCAM-1	**Vascular Cell Adhesion Molecule -1**
WHO	**World Health Organization**

ABSTRACT

The study aimed at investigating the relationship between blood group type and severe disease in P.falciparum malaria in three Ethiopian malaria endemic localities. A total of 210 cases of malaria (70 severe and 140 uncomplicated) and 190 healthy controls participated in the study. 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 the dominant blood type in both uncomplicated malaria (45.7%) and healthy controls (41.6 %). As compared to the uncomplicated malaria, a case of severe malaria was almost twice as likely to be of type A as to be of type O (O vs. A, odds ratio 0.42, 95% confidence interval 0.2007- 0.8789), and more than twice as likely to be of type B as to be of type O (O vs. B, odds ratio 0.38, 95% confidence interval 0.1638-0.8973), and in both cases the difference was statically significant (P=0.019 for O vs. A; P= 0.02 for O vs. B). Furthermore, individuals with severe malaria were about seven fold less likely to be of O as to be of type AB (O vs. AB, odds ratio 0.19, 95%; P=0.0005). There was significantly low mean hemoglobin concentration in the severe malaria patients than the uncomplicated malaria and healthy controls (P <.0001). Although the mean parasite density in individuals with severe malaria was higher than in those with uncomplicated malaria, the difference was not significant (P=0.35). Blood group O malaria patients had high mean hemoglobin concentration than those with blood group A, B, or AB, with significant difference only between blood group O and A (P=0.012), and blood group O and B (P=0.050). Furthermore, blood group O malaria patients were found to have a relatively low mean parasite density than patients with either blood group A or AB, but there was no significant difference in both cases (P=0.55 for O vs. A; P= 0.90 for O vs. AB) .The study revealed that on the basis of depressed hemoglobin concentration, patients with blood group O were less prone to severe P.falciparum malaria as compared to patients with blood groups A or B.

Key words: **Malaria, ABO blood group, Severe malaria, Uncomplicated malaria.**

1. Introduction

1.1 Malaria

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, there are approximately 515 million cases of malaria, killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa (Snow *et al*., 2005). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development.

The disease is caused by an obligate, intracellular protozoan parasite of the genus *Plasmodium*, transmitted to the vertebrate host by the female *Anophele* mosquitoes. Of the four species that infect humans (*P. falciparum, P. vivax, P. ovale* and *P. malariae*), *P. falciparum* is responsible for virtually all deaths (Ringwald *et al.,* 1993).

In Ethiopia, malaria is ranked as the leading communicable disease. Approximately 4-5 million cases of malaria are reported annually in Ethiopia (WHO, 2005). Malaria is found in about 75% of the total area of the country, and 40-50 million (>65%) of the total population is at risk of infection (WHO, 2005). Largescale epidemics occur every 5-8 years in certain areas due to climatic fluctuations and drought-related nutritional emergencies. There are also areas of stable transmission in some low-lying western regions of the country (WHO, 2005).

Infection with *P. falciparum* has a wide spectrum of manifestations classified as asymptomatic infection or clinical malaria. In malaria endemic areas, a significant proportion of individuals harbour parasites without presenting signs of clinical malaria (Rougemont *et al.,* 1991). Clinical manifestations of malaria range from mild uncomplicated to severe malaria. Fever accompanied by nausea, headache, malaise, cough, diarrhea and muscular pain are characterized as uncomplicated malaria. Severe anemia, cerebral malaria, renal failure, hypoglycaemia, pulmonary oedema, parasitemia,

convulsion and shock are the common complications of severe malaria (Qijun *et al*., 2000;
Menendez *et al*., 2000; May and Nicholas, 1999). While uncomplicated malaria is caused by all four *Plasmodium* infections, complicated malaria is mainly due to *P. falciparum* (Ringwald *et al.*, 1993).

The clinical outcome of malaria infection involves many factors that include: parasite factors (parasite load, multiplication rate, rosetting, cytoadherence or sequestration), host factors (immunity, age, receptors for rosetting and sequestration), and geographical and social aspects (Miller *et al*., 2002; Serghides *et al*., 2003). Due to changes in one or several of these factors, people will move from having an asymptomatic infection (presence of *P. falciparum* and absence of symptoms) to developing fever (symptomatic) to severe malaria and finally death.

Rosetting and sequestration are important elements of the pathology of all these different manifestations of malaria disease. In the host, effects of sequestration might include, a mechanical obstruction of infected red blood cells (iRBCs) in capillaries and a hindrance to microcirculatory blood flow, hypoxia, focal release of parasite toxins and inflammatory mediators, cerebral oedema and raised intracranial pressure (Chotivanich *et al.*, 1998; Miller *et al*., 2002; Sherman *et al*., 2003). Rosette formation also impairs blood flow, causing tissue ischemia and cell death (Serghides *et al*., 2003).

1.1.1 The life cycle of malaria

The life cycle of the protozoan parasite *P. falciparum* involves two hosts: female mosquitoes, exclusively of the genus *Anopheles* and humans. No animal reservoir or free living stages are known. Infection with *P. falciparum* occurs by injection of few sporozoites, perhaps between five and twenty, into the bloodstream of the human host during the blood meal of an infected female mosquito (Figure- 1).

Within minutes after entering the blood circulation, sporozoites are arrested in the liver and enter hepatocytes, where they multiply and develop into liver schizonts. After 5-14 days of parasite multiplication, the hepatocytes rupture and release thousands of

merozoites into the bloodstream, where they exclusively invade red blood cells. The intracellular parasites undergo maturation from ring stages to trophozoites to schizonts within 48 hours. Each schizont gives rise to 8-32 merozoites until the iRBC finally disrupts, thereby releasing the merozoites, which find their way into another erythrocyte within a few seconds, starting a new cycle of intraerythrocytic asexual replication. Often an adhesive behaviour is manifested by the iRBC some 16-20 hours into the intraerythrocytic cycle, when the parasite matures to the early trophozoite stage. At this stage, it expresses and transports molecular adhesins to the red cell surface, which are responsible for the sequestration of iRBCs to the deep vascular bed of organs or binding with uninfected red blood cells.

While propagation of intraerythrocytic parasites may persist for months, occasional ring stages develop into sexual forms of the parasite, termed micro- and macro gametocytes, which are infective for the female *Anopheles* mosquitoes. In the mosquito midgut, the gametocytes differentiate into gametes (1 macro- or 8 micro gametes, respectively) and, after fertilisation and zygote formation, transform into ookinetes. Each ookinete penetrates the midgut wall, develops into an oocyste and begins with sporogony: upon maturation, one oocyste contains thousands of sporozoites, which are released into the hemolymph. The sporozoites invade the salivary glands of the mosquito from where they are injected into a human host during the next blood meal.

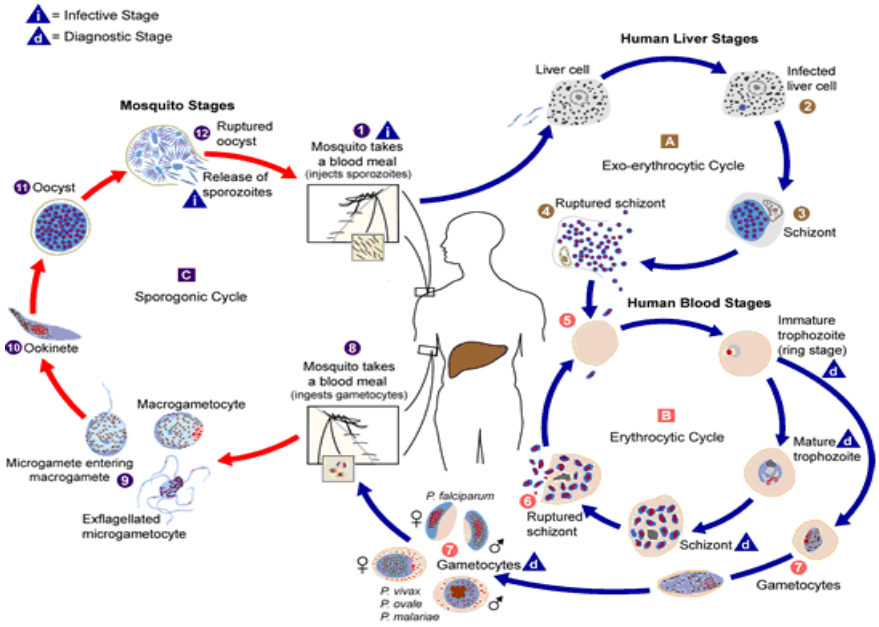


Figure 1: The Life Cycle of Malaria (CDC, 2006)

1.1.2 Immunity to malaria

There are several effectors of innate immunity against malaria. Some of these are macrophages, DCs, and NKT cells. The importance of macrophages in innate immunity to malaria is due to their ability to phagocytose infected erythrocytes in the absence of cytophilic or opsonizing malaria-specific antibodies. Studies by Serghides *et al*. (2003) indicated that CD36 is involved in this opsonin-independant phagocytosis of *P. falciparum*-infected erythrocytes by monocytes from non-immune individuals.

The importance of dendritic cells in innate immunity to malaria is due to their ability to stimulating NK cells activation. Although studies have revealed a role of DCs in immune response to many intracellular pathogens, relatively little is known concerning the role of DCs in immunity to malaria. Some studies have shown that *P. falciparum* infected red blood cells suppressed the maturation of DCs (Urban *et al*., 2003) and reduced their ability to activate T cells (Urban *et al*., 2003). However, studies using experimental animals have shown that DCs from infected mice are fully functional APCs (Perry *et al*., 2004).

NKT cells are important in pre-erythrocytic anti-malarial immunity. Once activated by TCR, NKT cells produce high levels of IFN-γ and IL-4 (Yoshimoto & Paul, 1994). This capacity may contribute them to exert anti-malarial immunity during blood stage where IFN-γ and IL-4 are involved in parasite clearance.

$CD4^+$ T cells play a crucial role in immunity against the asexual blood stages of the malaria parasite (Stephens & Langhorne, 2006). $CD4^+$ T cells produce cytokines which amplify innate immune response and down-regulate this response later on to avoid immunopathology. $CD4^+$ T cells are also required to help B cells to produce anti-malarial antibodies which are key components for parasite clearance. Protective immunity to blood stage of malaria infection is dependent on $CD4^+$ T cells together with antibodies and B cells (Stephens & Langhorne, 2006).

An infection with *P. falciparum* parasite induces elevated levels of both polyclonal (McGregor *et al*., 1971) and anti-malaria specific antibodies. It is evident that antibodies

are important in blood stage parasite clearance (Troye-Blomberg & Perlmann, 1988**)**. Protective anti-malarial antibodies belong to certain Ig classes and subclasses, with IgG being the most important isotype in this respect.

Malaria-specific antibodies mediate a number of anti-parasitic effector functions including: inhibition of cytoadherence and thus preventing sequestration of iRBCs and instead letting them be removed by spleen (David *et al*., 1983); inhibition of RBC invasion and antibody-dependent cytotoxicity (Kumaratilake & Ferrante , 1992). Anti-malarial antibodies are involved in the antibody-dependent cell-mediated inhibition (ADCI) mechanism where the parasite growth in inhibited by antibodies bound to Fc receptors on phagocytes. Antibodies may also facilitate parasite clearance by opsonization and phagocytosis by macrophages (Giribaldi *et al*., 2001).

1.2 Proposed biological mechanisms relating host ABO group to the pathophysiology of *P. falciparum* malaria

One of the major questions in malaria research is why infections with *P. falciparum* are often lethal as compared to other species. The possible explanation lies on additional mechanism s (rosetting and sequestration or endothelial cytoadherence), that have been highly developed by this parasite to evade immune response.

Several experimental works suggested that rosetting may be a parasite virulence factor because it causes microvascular obstruction (Miller *et al*., 2002; Sherman *et al*., 2003), which is thought to be a key process in the pathogenesis of severe malaria (White,1987). In an *ex vivo* model, *P. falciparum* rosettes were disrupted by high shear forces in the arterial side of the circulation but reformed in the postcapillary venules by adhesion of uninfected erythrocytes onto infected erythrocytes that were bound to endothelial cells (Miller *et al*., 2002; Sherman *et al*., 2003). This combination of rosetting and cytoadherence occurring simultaneously resulted in rosetting parasites causing greater obstruction to microvascular blood flow (Miller *et al*., 2002; Sherman *et al*., 2003).

Sequestration and rosetting involve a complex interaction between parasite-derived antigens that are expressed on the surface of iRBCs as proteins, and either one or more

receptors expressed on the surface of uninfected RBC, vascular endothelium or syncytiotrophoblasts in placenta (Beeson and Brown, 2004).

A number of parasite derived or modified proteins have involve in rosetting and endothelial cytoadherence were identified including *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), sequestrin and modified erythrocyte band 3 anion transporter, also termed as Pfalhesin (Ockenhouse *et al*., 1998).

Of all the above mentioned ligands, PfEMP-1 is the only parasite derived protein for which the full sequence information is available and it is the only adhesion mediating molecule, which have been shown by several laboratories to be parasite encoded and to be located on the iRBC surface. These adhesions mediate adherence to endothelial cells (sequestration, Hickey *et al*., 2000), to uninfected RBCs (rosetting, Fernandez *et al.*, 1988) and to other iRBCs (auto- agglutination, Chotivanich *et al.*, 1992).

The structure of PfEMP-1 includes variable numbers of 2 types of adhesive binding domains: Duffy binding-like (DBL) regions and cysteine-rich interdomain regions (Smith *et al.*, 2001). DBL-1α demonstrates lectin-like properties, causing it to bind primarily to cells bearing A and B blood group oligosaccharides, and to other glycosylated targets, such as the glycoprotein CD35 (CR1), and heparan sulfate–like glycosaminoglycan. (Chen *et al*., 2000). Cysteine-rich interdomain region-1, however, binds principally to CD36 (platelet glycoprotein IV), thus targeting platelets and endothelium.

A number of receptors expressed on the surface of endothelial cells and uninfected RBCs have been shown to bind erythrocytes infected with *P falciparum.* Thrombospondin (TSP) (Reeder *et al.*, 1999), CD36 (Barnwell *et al.*, 1989), ICAM-1 (CD54) (Berendt *et al.*, 1989), VCAM-1 (CD 106) (Ockenhouse *et al.*, 1992), E- selectin (Ockenhouse *et al.*, 1992), chondroitin sulfate A (CSA) (Buffet *et al.*, 1999), and PECAM-1 (CD31) (Treutiger *et al.*, 1997) are some of the endothelial receptors which mediate cytoadherence (Figure2).

The ABO determinants are present on endothelial cells and platelets as well as erythrocytes, and it seems likely that parasite isolates that bind to A or B determinants on

erythrocytes to form rosettes may also bind A or B antigens on other cell types, which could enhance sequestration and increase pathogenic potential (Cserti and Walter, 2007). Furthermore, as possible receptors on the surface of uninfected red blood cells, i.e. receptors mediating rosetting, blood group antigen A and B (Chen , *et al*., 2000) CD36 (Handunnetti *et al.*, 1992), complement receptor 1 (CR1, CD35) (Rowe *et al.*, 1997) and heparan sulfate (HPS) (Chen *et al.*, 1998), were described.

Due to expression of blood group antigen A and B in group A and B individuals and the suggested role of these blood group antigens in rosetting and sequestration, several researchers tried to investigate whether individuals having non-O blood groups are more prone to severe malaria as compared to individuals with group O .However, findings from the various studies that investigated the relationship between ABO blood group and severe *P.falciparum* malaria were inconsistent and contradictory.

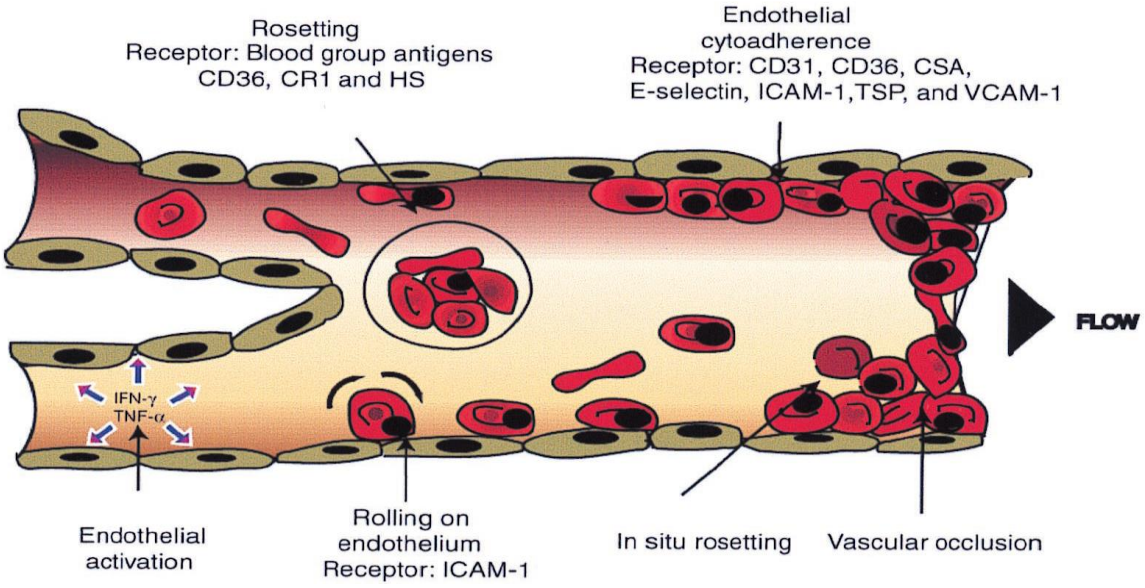


Figure 2: The processes of cytoadherence and rosetting in postcapillary vasculature & receptors and ligands which are involved in the two processes (Chen *et al.*, 2000)

Parasitized RBCs expressing cysteine-rich interdomain region-1 can tether and roll on endothelial adhesion molecules, with final direct adhesion to CD36. In addition to direct endothelial binding, infected RBCs may also bind to platelets, in turn adhering to endothelial cells. Platelet-mediated RBC adhesion may be especially relevant to cerebral malaria because CD36 is not strongly expressed on cerebral endothelial cells (Silamut *et al*., 2006). Platelet-mediated RBC adherence involves VWF .VWF is released from endothelial storage sites in response to endothelial damage or activation by cytokines found in individual with malaria (Shi., *et al*., 2004). Hollestelle *et al.* (2006) have shown that cerebral malaria is associated with increased levels of VWF and blood group O individuals have lower plasma VWF levels than non-O individuals. As a result, the consistently lower levels of VWF found in group O subjects provide an additional selection advantage among group O individuals infected with *P falciparum*.

1.3 ABO blood group and P. *falciparum* malaria

Studies on the relationship between the ABO blood group and *P. falciparum* malaria suggests that the association between the two is still debatable and yet to be well defined. While some studies reported no significant association between *P. falciparum* malaria and the ABO blood group, other studies clearly indicated the existence of a significant association between the two. Factors investigated by the various authors, in association with ABO blood group, included malaria prevalence (Bayoumi *et al*., 1986; Montoya *et al*., 1994; Uneke *et al*., 2006), parasitemia (Martin *et al*., 1979; Kassim and Ejezie, 1982), and parasitemia/parasite antibody titer (Akinboye and Ogunrinade, 1987; Thakur and Verma, 1992).

In Columbian, Montoya *et al*. (1994) studied the relationship between erythrocyte antigens and the presence of malaria infection by *P. vivax* and *P. falciparum* in four different ethnic groups of two Departments of Colombia. Although malaria infection by *P. falciparum* was found in 91.4% of malaria-infected blacks, they observed no significant differences between the presence of malaria infection and ABO antigens. In central Sudan, Bayoumi *et al*. (1986) reported absence of association between the ABO blood group and *P. falciparum* malaria. They examined adults claiming resistance to malaria who were identified in the Sennar region of central Sudan, where *P. falciparum* is

hyperendemic but seasonal in transmission and found no association with ABO blood groups.

In contrast to these reports, there are several studies that reported a significant association between ABO group distribution and *Plasmodium falciparum* malaria. Fischer *et al.* (1989) compared 209 mild malaria outpatients and 280 severe malaria inpatients in Zimbabwe. They suggested that group O hosts of *P.falciparum* malaria tend to have mild disease and less clinical outcome than A hosts. Coma was three times as likely to be group A as O. Because patients with coma are at a high risk for death, this study supports the hypothesis that group O individuals may have a survival advantage in severe malaria.

Pathirana *et al*. (2004) compared 243 (163 mild and 80 severe) *P.falciparum* malaria cases and 65 patient with severe non -malaria illness. Severe malaria patients appeared to have severe anemia, cerebral malaria and multiple organ dysfunctions including renal and pulmonary failure. The proportion of group O in mild malaria cases was 48%, but was only24% in severe malaria cases. In striking contrast, the proportion of group A in mild cases was 25%, but was 33% in severe cases. The distribution of ABO group was highly statistically different in severe malaria syndromes compared with uncomplicated malaria or the control population. Once again, a case of severe malaria was nearly 3-times as likely to be group A as O. This study provided the strongest statistical evidence of an association between ABO and disease severity in *P falciparum* infection.

Lell *et al*. (1999), assessed 200 cases (100 severe and 100 mild) of *P. falciparum* malaria in Gabon. Patients who were positive for *P. falciparum* malaria and positive for at least one of the following signs and symptoms of severe malaria were considered as severe cases. These are severe anemia, cerebral malaria, hyperparasitemia and clinical signs of severe malaria. The ratio of group A to group O in patients with severe malaria was 0.50, but was only 0.17 among those with mild malaria (3-fold relative risk).Among all group A individuals, 71% had severe malaria and only 29% had mild malaria . In contrast, among all group O cases 46% had severe malaria and 54% had mild malaria.

Loscertales *et al*. (2006), reported data collected before the HIV pandemic on pregnancy outcomes among women infected with *P falciparum* in The Gambia. Subjects of interest

were pregnant women with no evidence of malaria infection of the placenta. Among 89 primiparae, 41 had active placental parasite infection. In this subset, there was a significantly higher birth weight of children born to group O mothers compared with non-group O mothers. Infant length and placental weight were greater, and placental parasite count was lower among group O mothers compared with non-group O, although these values did not reach statistical significance, perhaps because of the small sample size. A larger study was recommended to confirm any association between birth outcomes and ABO blood group among women with *P falciparum* infection during pregnancy. Nevertheless, this study was interesting because it suggests that a group O survival advantage in *P. falciparum* infection may begin in utero during fetal development.

However, most of the studies which investigated the association between P.*falciparum* malaria and ABO blood group used only parasitemia (parasite density) as criteria to group patients under the severe and mild malaria categories. Others have suggested that clinical severity, rather than incidence or prevalence of detectable parasitemia, is a more relevant outcome to assess ABO group and survival (Cserti and Walter, 2002). Studies reporting both clinical features (such as cerebral malaria) and laboratory markers (such as level of parasitemia), carry more weight than those reporting only laboratory markers such as level of parasitemia, because the later doesn't always predict survival (Cserti and Walter, 2002). Furthermore, it was also pointed out that many of the studies that investigated the association of ABO blood group and malaria were flawed by absent or inappropriate healthy controls (Christine *et al*., 2007).

Due to the absence of control group and their focus only on laboratory markers than clinical severity these studies failed to identify any association between ABO blood group and disease outcomes from *P. falciparum* infections. Therefore performing a study by avoiding defects in previous studies, the present investigation aims to show difference in disease severity between malaria patients with different ABO blood groups in Ethiopian populations.

This is justifiable by the fact that rosetting frequencies of blood group A, B, AB and O are not the same due to *P. falciparum* strains of different countries. For instance, Rosette

formation was more common with group A and, to a lesser degree, group B blood using malaria strains from The Gambia (Udomsangpetch *et al*., 1993), with groups A and AB blood using Kenyan strains (Rowe *et al* ., 1995), with group A blood using strains from Uganda and one strain from Thailand (Carlson and Wahlgren, 1992), and with group B blood using other strains from Thailand (Carlson and Wahlgren, 1992). This shows that it is inappropriate to make a firm statement on whether blood group, via its influence on rosette formation, is causally associated with severe malaria by using *P. falciparum* strains of few countries. And further *in vitro* studies of rosette formation using similar strains of *P. falciparum* is useful to test whether the finding is similar with the clinical data of ABO groups and risk of severe *P. falciparum* malaria as well as to make a firm statement on whether blood group, via its influence on rosette formation, is associated with severe malaria. Therefore, the present study aims at providing more data on ABO blood groups and the risk of severe *P. falciparum* malaria infections in Ethiopia.

2. OBJECTIVES OF THE STUDY

General objective

To determine whether severity of disease due to the Ethiopian strain of *Plasmodium falciparum* is associated with ABO blood group types.

Specific objectives

1. To determine which, if any, of the ABO blood group types is less prone to severe disease in patients infected with *P. falciparum* in Ethiopia.
2. To determine the effect of ABO blood group type on anemia in *P. falciparum* malaria infection.
3. To determine the effect of ABO blood group type on parasite density in *P. falciparum* malaria infection.

3. MATERIALS AND METHODS

3.1 The Study Areas

The study was conducted in Nov, 2007 –Jan, 2008 in Ziway, Awash and Methara areas. Ziway is found in Oromia Regional state, East Showa Zone, Adamitulu Judo Kombolcha Woreda (Figure: 3). It is 163 Km south of Addis Ababa alongside of Lake Ziway. The rainfall pattern is similar to other Ethiopian regions with the main rainy season starting in June and extends up to August/September while the short rainy season begins in March and extends to April/May but usually it is very erratic. The area has a fairly warm climate with mean annual temperature of 20^{O} C and annual maximum and minimum temperature of 27.5 ^{O}C and 13.9^{O}C respectively .The mean annual rain fall of the Ziway area is 13.7 mm. The area is characterized by lowland marks with sparsely distributed *Acacia* trees and thorny bushes.

Awash is a market town in central Ethiopia. Located in Administrative Zone 3 of the Afar Region (Figure.3). Awash lies on the Addis Ababa-Djibouti Railway (http://en.wikipedia.org). Based on figures from the Central Statistical Agency in 2005, Awash has an estimated total population of 11,053, of whom 5,748 were males and 5,305 were females (CSA, 2005).

Metehara is a town in central Ethiopia. Located in the Misraq Shewa Zone of the Oromia Region (Figure. 3), this town has a latitude and longitude of 08°54′N, 39°55′E with an elevation of 947 meters above sea level (http://en.wikipedia.org). It has an estimated total population of 21,348 of whom 10,763 were men and 10,585 were women (CSA, 2005).

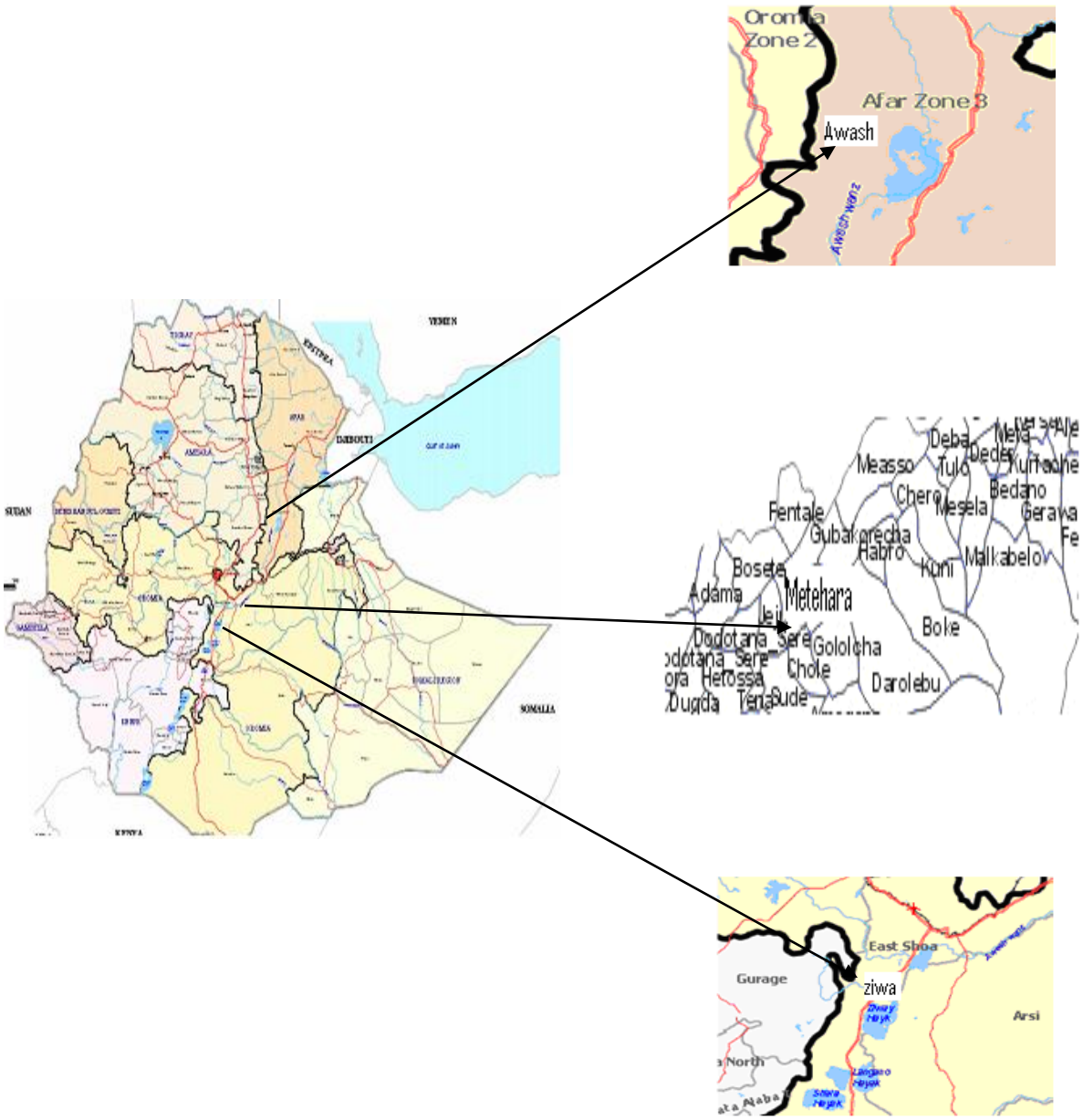


Figure 3. Geographical location of the study sites (http://www.telecom.net.et).

3.2 Study design

A health facility-based cross-sectional study was carried out in Oromia and Afar regions of Ethiopia between November 2007 and January 2008 to determine the association of ABO blood group and *P. falciparum* malaria. The laboratories were chosen as study sites because most of the malaria cases in Ethiopia are found in these regions and assumed that they represent the areas.

All cases of *P. falciparum* malaria who were admitted to any of the five health centers in east shoa (Jido health center in Ziway, Metehara health center in Methara, Tena Amba health center in Bulbula, and Bulbula health center, in Bulbula) and one health center in Afar region (Awash health center, in Awash) between November 2008 and January 2008 were considered as potential subjects. The required sample size was equally divided among the two regions.

Patients who had received antimalarial treatment in the 48 hours prior to the microscopical/RDT confirmation of their malaria, and those co-infected with P. *falciparum* and other species of *Plasmodium* were excluded from the study. Patients critically ill and unable to respond for the interview were also excluded from the study.

3.3 Sample size determination

A total of 400 were expected to be included in the study considering 95% confidence interval, and 5% marginal error, and non response rate 10%. Since there are no previous epidemiological data in Awash and Metehara 50% prevalence was taken for each and 17 % prevalence was taken for Ziway (Yihenew, 2007, M.Sc Thesis). Then by taking the average of these three cases the sample size was finally determined to be approximately 400 samples (individuals).

Total study subjects = $\frac{z^2 p (1-p)}{d^2} = 364$ (Danial, 2005)

= 400 (With 10% non- response rate)

Where p= Mean malaria prevalence of study areas= 39 %

Z= Confidence interval=95%= 1.96

d = Marginal error= 5% = 0.05

3.4 Socio demographic data

A pre-tested structured questionnaire were administered requesting demographic information on age, sex, length of residence in the area and anti malarial drug treatment before 48 hours. All patients fulfilling the inclusion criteria were interviewed at an exit from the laboratory after completing their diagnosis and treatment services. The interview was continued without interruption until the required number for each laboratory was completed.

3.5 Clinical data

Data of cerebral malaria, circulatory collapse, and other signs and symptoms of severe malaria were collected by medical practitioners. The clinical data were used to classify patients into the severe and uncomplicated malaria categories.

3.6 Blood collection and processing

After verbal consent, a drop of blood sample from the finger or toe of the study subjects was collected. The collected blood sample was used for thick and thin blood film preparation, hemoglobin measurement, ABO blood group typing and other analyses.

3.6.1 Rapid diagnostic test for P.*falciparum*

Paracheck Pf is a rapid test for detection of *P.falciparum*. The test is used for the detection of *P.falciparum* only. The procedure for the detection of *P.falciparum* is summarized as follow: gently a drop of fresh whole blood was allowed to touch the test area of the strip. Then five drop of clean buffer were added so that the blood diffuses slowly along the pad. At the end of 15 minutes the result is read as indicated in figure 4.

Figure 4: Paracheck Pf in identification of malaria parasite (the mark A indicates the area where blood is applied and B indicates where buffer is added) (Orchid biomedical systems, Verna industrial estate, verna, India).

3.6.2 Thick and thin blood film preparation

Both thick and thin blood smears were prepared in a same film slide. Thick blood smears were directly stained using Giemsa method for 20 minutes, while thin blood smears were initially fixed with methyl alcohol for a few seconds and then stained for 20-30 minutes. Subsequently, clean water was poured on the surface of stained films for 10–15 seconds and then the films were dried. The slide was examined under an ordinary light microscope. The result was considered negative when *P.falciparum* was not found in the blood smear or exists with other *Plasmodium* species, and positive if there was any.

3.6.3. Determining parasite density in the blood film

Malaria parasites were counted on Giemsa-stained thick blood films per 200 white blood cells (WBC). Parasites and leukocytes were counted in the same fields until 200 leukocytes were counted. This counting was done by hand tally counter. Parasite densities were estimated using an assumed leukocyte count of 8000 leukocytes/µl blood. The number of parasite per microliter of blood was calculated by multiplying the number of parasite (asexual stages) counted by 8000 leukocytes and dividing the product by 200 (Cheesebrough, 1998).

$$\text{Parasite } /\mu l = \frac{\text{WBC}/\ \mu l \times \text{parasite counted against 200 WBC}}{200}$$

Where WBC is total white cell count and assumed to be 8000 leukocytes/µl for each individual.

3.6.4 Hemoglobin determination

For analysis of hemoglobin concentration, 5 µl (approximately 1 drop) blood was collected directly from the finger by capillary action into the Hemocue microcuvettes and was then analyzed directly by Haemocure haemoglobinometer (HemCue AB, Angelhom, Sweden).

3.6.5 ABO blood group typing

The ABO blood type of each subject was determined by the reaction of erythrocytes with antisera for blood group A and B (De Zoysa, 1985).The procedure is summarized as follows: Two drops of each blood sample was placed in two different places of a grease-free clean glass slide. Thereafter, antisera for blood group A or B was added in each blood sample. Based on the reaction (agglutination) of erythrocytes with antisera for blood group A and B, blood type of each subject was determined.

3.7 Ethical clearance

Informed consent was obtained from all the adult patients and from the parents/guardians of the children. The study protocol was approved by the ethical committee of the department of Biology, Science Faculty, Addis Ababa University. Positive individuals were treated according to standard guideline for malaria (MOH, 2004b). Lastly, information and education was given to some of the study participants with regard to malaria prevention, control, and early diagnosis and adequate treatment.

3.8 Data analysis

All raw data generated from this study was entered into Microsoft Excel database system and referenced with location. This included data on age, sex, length of residence in the area, anti malarial drug treatment before 48 hour, parasite count, hemoglobin level blood group type and other.

Statistical analyses were performed using SPSS 13.0 and SISA software. Data were analyzed by the use of Chi-square test (χ^2) for association and one way ANOVA for comparing means. Values were considered to be statistically significant when P-values are less than or equal to 0.05.

4. RESULTS

4.1 Description of the study participants

During the study period, a total of 400 blood samples were collected from individuals living in Ziway, Awash or Metehara. Of these, 70, 140, 190 were with signs and symptoms of severe malaria, with uncomplicated malaria and healthy controls, respectively (Table1).

There were 37 (57%) males and 33(48%) females in the SM category. Out 140 uncomplicated malaria patients, there were 70 (50%) males and 70 (50%) females (Table1). Individuals suffering from the most serious symptoms were on average only 14.3 years old, and younger than individuals in uncomplicated malaria (15.6 yrs.) and healthy control (15.0 yrs.) categories. However, there was no significant difference in mean age between the three cases (*P*=0.80) (Table1). Individuals with severe malaria harboured higher mean asexual forms of *P. falciparum* per microlitre blood (mean (±SD) = 4166 (3551)) than individuals with uncomplicated malaria (mean (±SD) = 3418 (6200)).However, the difference in parasite density between the asymptomatic individuals and those experiencing a severe course of malaria was not significant (*P*=0.35) (Table1). The mean (±SD) hemoglobin concentration was 10. 4 (4.3) gm/dl, 12.0 (2.4) gm/dl and 12.8 (2.41) gm/dl for severe malaria, uncomplicated malaria and healthy control cases, respectively. And the difference in mean hemoglobin concentration between the severe malaria, uncomplicated malaria and healthy control cases was statistically significant (P< 0.0001) (Table1).

Table 1: Parasite density and hemoglobin concentration among severe and uncomplicated malaria patients from Ziway, Awash and Metehara, Ethiopia, Nov, 2007-Jan, 2008

Category	***Total* (N)**	**Male**	**Female N (%)**	***Average age, (years)*** **	***Mean Hb, (g/dl)*** [X]	**Mean Parasite density per µl of blood** [X]
Severe malaria	70	37(53%)	33(47%)	14.3 [4-16.9]	10.4(4.3)	4166 (3551)
Uncomplicated malaria	140	70(50%)	70(50%)	15.6 [6.5-18.4]	12.0(2.4)	3418 (6200)
Healthy control	190	86(45%)	104(55%)	15.0 [6.9-18.4]	12.80 (2.41)	NA[≠]
***P*- value**[†]	NA[≠]	NA[≠]	NA[≠]	0.80	<.0001	0.35

* Significant difference

[X] Values shown in bracket are the ± standard deviation

† ANOVA

≠ NA, not applicable

** Values shown in bracket are the Range

4.2 Hemoglobin concentration between male and female study participants

Hemoglobin concentration between male and female study participants is shown in Table 2. During the study period, there were 193 (48%) males and 207 (52%) females (Table2). There was higher mean hemoglobin concentration in male study subjects than females, however, the difference was not statistically significant (P=0.25).

Table 2: Comparison of hemoglobin concentration between male and female study participants from Awash, Metehara and Ziway, Ethiopia, Nov, 2007-Jan, 2008

Sex	**Total (%)**	**Mean Hb, *(g/dl)*** [x]
Male	193 (48%)	11.9 (4.63)
Female	207 (52%)	11.5 (3.18)
P -value	NA[≠]	0.25

[x] Values shown in bracket are the ± standard deviation

≠ NA, not applicable

4.3 The frequency of blood group A, B, AB and O between the severe malaria and uncomplicated malaria or healthy controls.

The frequency of blood group A, B, AB and O between the severe malaria and uncomplicated malaria or healthy controls are shown in Table 2. As compared to the uncomplicated malaria cases, cases of severe malaria were almost two times less likely to be of blood group O (OD=0.35, P=0.0013), and 1.38 times more likely to be of blood group B (OD=1.38, 95% confidence interval 0.671-2.864) (Table 3). Furthermore, individuals with severe malaria were about 4 fold more likely to be of blood group AB than those with uncomplicated malaria (OD=4.06, P=0.0005) (Table 3).as compared to healthy controls, the cases of severe malaria were twice more likely to be of O (OD=0.42, P=0.35) (Table 3).

Table 3: Frequency of each ABO blood group type among the severe malaria (SM**)** and uncomplicated malaria (UM) or healthy controls (HC) from Ziway, Awash and Metehara, Ethiopia, Nov,2007-Jan, 2008

Blood group compared	**SM vs.UM** [X]	**SM vs. HC** [X]
A vs. (B,AB &O)	1.29 [0.705-2.381] (0.4)	1.44[0.80-2.57] (0.40)
B vs. (A,AB &O)	1.38 [0.671-2.864] (0.37)	1.21[0.61-2.38](0.37)
AB vs. (A,B &O)	4.06 [1.747-9.468] (0.0005)	0.68[0.87-3.76] (0.0005)
O vs. (A,B &AB)	0.35[0.183-0.673] (0.0013)	0.42[0.22-0.78] (0.35)

[X] values shown are the odds ratio, [95% CI],(P-value)

4.4. The frequency of ABO blood group types in the severe malaria, uncomplicated malaria and healthy controls

The odd ratios and P values for the frequency of O and non-O blood group types in the severe malaria, uncomplicated malaria and population control cases are shown in Table 4**.** A case of severe malaria was almost twice as likely to be of type A as to be of type O (O vs. A, odds ratio 0.42, 95% confidence interval 0.20- 0.88), and more than twice as likely to be of type B as to be of type O (O vs. B, odds ratio 0.38, 95% confidence interval 0.16-0.89), than patients with non severe malaria (Table 4). Furthermore, individuals with severe malaria were about seven fold less likely to be of O as to be of type AB (O vs. AB, odds ratio 0.19, 95% confidence interval 0.07-0.51) (Table 4).

As compared to healthy controls, a case of severe malaria was more than twice as likely to be of type A as to be of type O (O vs. A odds ratio 0.42, 95% confidence interval 0.21-0.88), almost twice more likely to be of type B as to be of type O (O vs. B odds ratio 0.47 , 95% confidence interval 0.21-1.06), and thrice more likely to be of type AB as to be of type O (O vs. AB odds ratio 0.33, 95% confidence interval 0.14-0.78) (Table 4).

Table 4: The odds ratios and P values for the frequency of O and non-O blood group types between the three study categories: patients with severe malaria (SM), uncomplicated malaria (UM), and healthy controls (HC) from Ziway, Awash and Metehara, Ethiopia, Nov, 2007-Jan, 2008

Blood group compared	***SM vs. UM*** [X]	***SM vs. HC*** [X]	***UM vs. HC*** [X]
O vs. A	0.42, [0.20-0.88],(0.019)	0.43, [0.21-0.88], (0.02)	1.02,[0.60-1.72] ,(0.93)
O vs. B	0.38, [0.16-0.89],(0.02)	0.47,[0.21-1.06], (0.07),	1.23,[0.66-2.29],(0.50)
O vs. AB	0.19, [0.07-0.51] , (0.0005)	0.33,[0.14-0.78],(0.009)	1.69, [0.76-3.73],(0.19)

[X] values shown are Odds ratio, [95% CI], (P-value)

4.5. Mean hemoglobin concentration and parasite density in the severe malaria patients

Parasite density and hemoglobin concentration were compared between blood group O and A, B or AB severe malaria patients to determine the protective effect of blood group O on parasite density or hemoglobin concentration. There was a significantly higher hemoglobin concentration in blood group O than group A (P= 0.012) or B (P=0.050) severe malaria patients (Table 5). Blood group AB severe malaria patients also had low mean hemoglobin concentration than those with group O, the differences in mean hemoglobin concentration was , however, not significant (P=0.77) (Table 5).

Blood group O SM patients had high mean parasite density (mean, (±SD) =3905.00, (3261.27)) than patients with group B, (mean, (SD) =3405.33,(2514.09)) , but the difference was not statistically significant (P= 0.63). In contrast, the mean parasite density was low in blood group O patients (mean, (SD) =3905.00,(3261.27)) as compared to patients with blood group A (mean, (SD) =4684.80,(4399.17)) or AB (mean, (SD) =4057.14,(3430.60)), however, there was no significant difference in parasite density between blood group O and A(P=0.55) as well as blood group O and AB (P=0.90) cases(Table 5).

Table 5: Comparison of hemoglobin concentration and parasite density between blood group O and group A, B and AB in severe malaria patients from Awash Metehara and Ziway, Ethiopia, Nov, 2007-Jan, 2008

Blood group compared	**Total, n (%)**	**Mean Hb, g/dl** [X]	**Mean Parasite density per µl of blood** [X]
O	16 (22.9%)	12.39 (4.64)	3905.00(3261.27)
A	25 (35.7%)	8.93 (3.73)	4684.80(4399.17)
P- value†	NA[≠]	0.012*****	0.55
O	16 (22.9%))	12.39(4.64)	3905.00(3261.27)
B	15(21%)	9.43(3.23)	3405.33(2514.09)
P -value†	NA[≠]	0.050*****	0.63
O	16 (22.9%)	12.39 (4.64)	3905.00(3261.27)
AB	14 (20%)	11.89 (4.82)	4057.14(3430.60)
P- value†	NA[≠]	0.77	0.90

[X] values shown in bracket are the standard devasion

+ ANOVA

*****Significant difference

[≠] NA, not applicable

4.6 ABO blood group characteristics of the study participants

Of the 70 blood samples examined 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 (Figure 5a). Out of the total uncomplicated malaria cases, 42 (30%), 23 (16.4%), 11 (7.9%) and 64 (45.7%) had blood group A, B, AB and O, respectively (Figure 5b).Similarly, the proportion of group O in population control category was 41.6 %, but was only 27.9 % for blood group A (Figure 5c). There were low percentage of Blood group O patients in the SM category than in both uncomplicated malaria and healthy control cases. Blood group O presented in only 22.9 % of severe malaria patients compared with 45.7% and 41.6 % of the uncomplicated and PC cases, respectively (Figure 5).

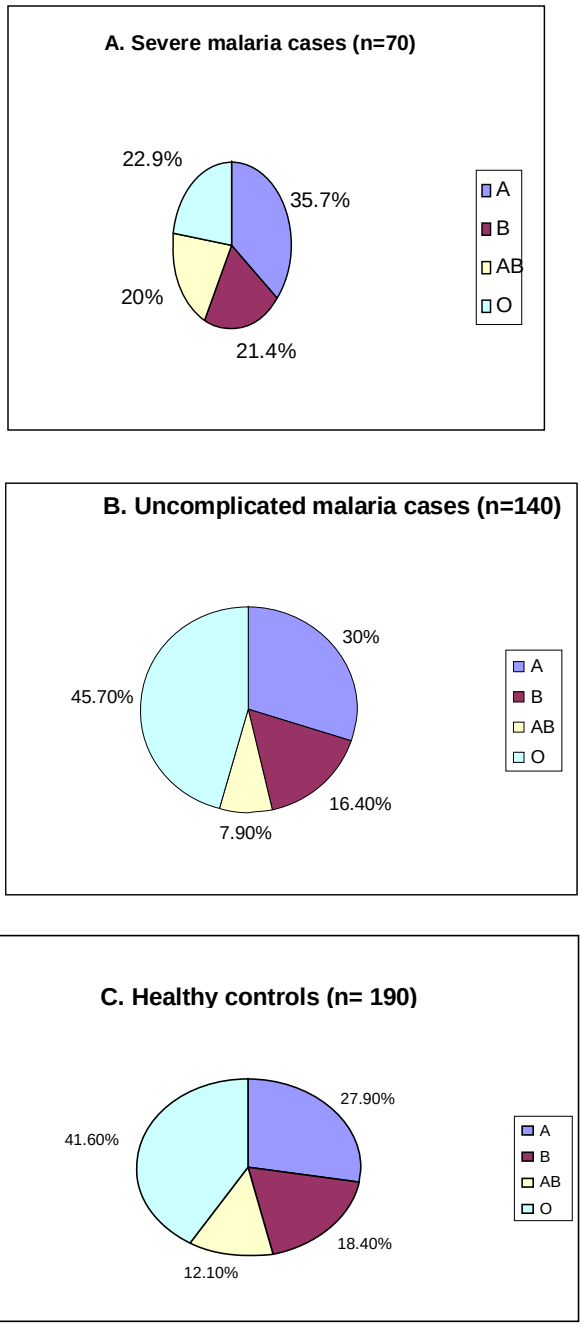


Figure 5. Percentage distribution of the ABO blood group in the severe malaria, uncomplicated malaria and healthy controls categories. (*A*) Distribution of ABO blood group frequencies in severe malaria cases (*B*) Distribution of ABO blood group frequencies in uncomplicated malaria controls, (*C*) Distribution of ABO blood group frequencies in healthy controls. Percentages are shown within each section of the pie from Ziway, Awash and Metehara, Ethiopia, Nov, 2007-Jan, 2008

4.7 Features of severe *P. falciparum* malaria in the study participants

The clinical, laboratory and parasitological characteristics of the 70 individuals with severe malaria are shown in Figure 6. Parasitemia (high parasite density) and circulatory collapse were the commonest features of severe malaria present in 16 (22.9%) and 20 (28.6%) of the patients. Severe anemia and cerebral malaria occurred in 14 (20%) and 5 (7.1%) of the patients, respectively. Furthermore, there were 4 (5.7%) cases of severe anemia and parasitemia, 3(4.3%) cases of severe anemia and circulatory collapse, 3(4.3%) cases cerebral malaria and circulatory collapse, 3(4.3%) cases of circulatory collapse and parasitemia and 1(1.4%) case of severe anemia, circulatory collapse and cerebral malaria (Figure 6).

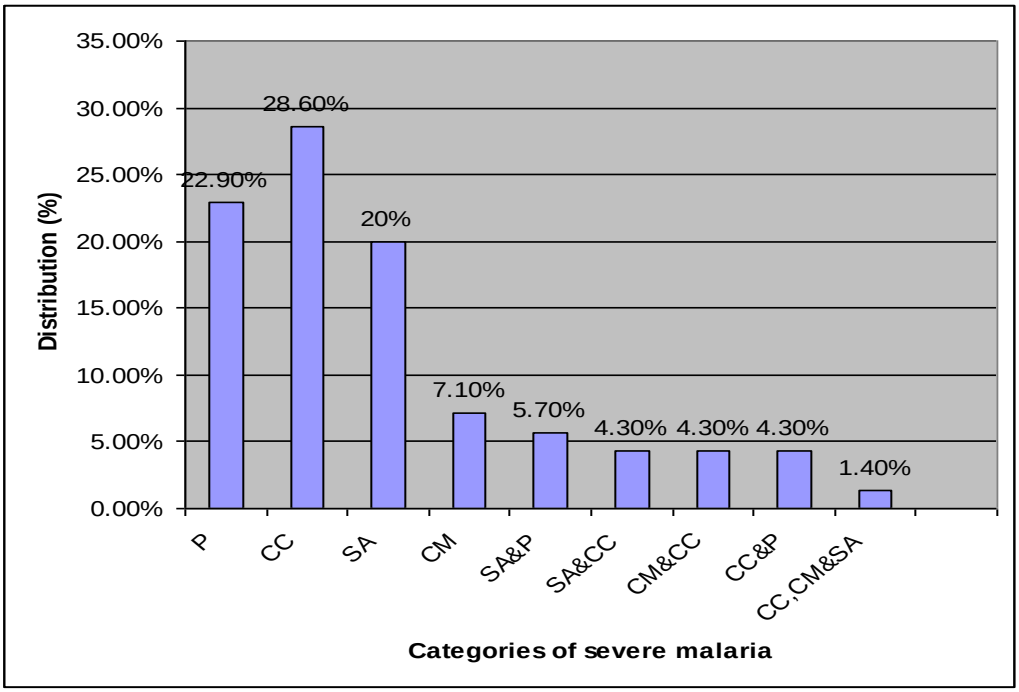


Figure 6: Features of severe malaria in the study participants (n=70) from Ziway, Awash and Metehara, Ethiopia, Nov,2007-Jan, 2008

Abbreviations: CM=Cerebral malaria SA=Severe anemia

C.C= Circulatory collapse P= Parasitemia

5. DISCUSSION

In the present study individuals with severe malaria have lower hemoglobin concentration than those with uncomplicated malaria or healthy controls. This is in agreement with similar study in Mali (Rowe *et al*., 2007), who reported significantly lower hemoglobin concentration in patients with severe malaria than uncomplicated malaria or healthy controls. The basis for malaria mediated anemia has been elucidated by several investigators (Carlson *et al*., 1990; Rowe *et al*., 1995; Hotez *et al*., 2006); Carlson *et al*. 1990 and Rowe *et al*. 1995 suggested that the virulence of *P. falciparum* is associated with the capacity of the infected RBC to adhere to uninfected RBCs, a process known as resetting. Hotez *et al*. (2006) also suggested that malaria causes anemia by increased distraction of both parasitized and non parasitized red blood cells or by dyserythropoiesis resulting from host production of inflammatory cytokines. Therefore, the difference in mean hemoglobin concentration between the severe malaria and uncomplicated malaria or healthy control cases may be due to existence of increased distraction of both parasitized and non parasitized red blood cells and higher rosetting in severe malaria patients than those with uncomplicated malaria and healthy controls.

On contrary to the report of Rowe *et al*. (2007) of significantly higher parasite count in patients with severe *P. falciparum* malaria than with uncomplicated malaria, the present study did not reveal individuals with severe malaria to have significantly higher parasite count than patients with uncomplicated malaria. However, this result does not tell us that parasite load can't be the reason for variation in hemoglobin concentration between the severe malaria, uncomplicated malaria and healthy control cases. Because absence of variation in parasite density between severe and uncomplicated malaria patients in the present study may be due to higher sequestration in severe malaria than uncomplicated malaria cases. Therefore, it is reasonable to recommend the development of other tool (method) that determines the exact parasite density without the influence of sequestration.

As compared to the uncomplicated malaria patients, the cases of severe malaria were less likely to be of blood group O than A, B, or AB. This result substantiated the fact that blood group O protects against severe malaria as compared to other blood groups. This was in agreement with other studies (Lell *et al*., 1999; Pathirana *et al*., 2005). On the other hand, a number of other studies (Martin *et al*., 1979; Kassim and Ejezie, 1982; Akinboye and Ogunrinade, 1987; Thakur and Verma, 1992) have reported that blood group O does not protect against severe malaria as compared to other blood groups.

The mechanism by which blood group O confers somewhat protective effect against severe malaria compared to blood groups A, B, and AB is not totally understood. However, a logical explanation may be provided on the basis of rosette formation by pRBCs. Some studies have clearly established that parasitized erythrocytes form rosettes more readily with IRBCs of either A, B, or AB blood groups than with those belonging to blood group O (Udomsangpetch *et al*., 1989, 1993; Carlson and Wahlgren, 1992; Rowe *et al*. ,1995; Barragan *et al*., 2000). Also, it is well established that this parasite-triggered RBC rosette formation is associated with the severity of clinical disease (Rowe *et al*., 1995) .This may explain why severe malaria was more common in blood group O than other blood group.

Unlike the present study, most of the studies which reported the reduced (lower) association between blood group O and severe malaria used only parasitemia (parasite density) as criteria to group patients under the severe and uncomplicated malaria categories. However, parasitemia not always predict survival since a low percentage of circulating parasitized red cells may either reflect less severe infection (good prognosis), or a greater degree of adhesion of parasitized cells to the vascular endothelium (bad prognosis) (Gravenor *et al*., 1998; Hearn *et al*., 2000). Therefore, inappropriate grouping of patients under the severe malaria and uncomplicated malaria categories by using only parasite density (parasitemia) may have been responsible for the conflicting reports on the relative protective effect of blood group O against *P. falciparum* severe malaria.

In the present study, it is shown that the cases of severe malaria were significantly more likely to be of blood group AB than blood group A, B or O. This was consistent with

study in Sri Lanka (Pathirana *et al*., 2005), where severe malaria patients were significantly more likely to be of group AB.

Previous studies had suggested that sequestration is higher in patients with blood group A, B and AB than in those with blood group O (Jenkins and O'Donnell, 2006; Cserti and Walter, 2007). In the host, effects of sequestration might include a mechanical obstruction of infected red blood cells (iRBCs) in capillaries and a hindrance to microcirculatory blood flow, hypoxia, focal release of parasite toxins and cerebral malaria (Chotivanich *et al.*, 1998; Miller *et al*., 2002; Sherman *et al*., 2003). Therefore, higher sequestration in blood group AB individuals may make severe malaria cases to be more likely blood group AB.

Although blood group O severe malaria patients appeared to have higher hemoglobin concentration than blood group AB, the present study didn't reveal individuals with group O to have significantly higher hemoglobin concentration than those with group AB. This is in agreement with similar study in Zimbabwe (Fischer and Boone, 1998), who reported the absence of significant difference in hemoglobin concentration in patients with blood group O than AB. Furthermore, there was no significant difference in parasite density between blood group O and AB severe malaria patients. Since parasite density was suggested to be one of the causes of anemia in malaria patients, the absence of significant difference in parasite density between blood group O and AB patients may have been responsible for the absence of variation in hemoglobin concentration between the two groups.

Blood group O severe malaria patients had significantly higher hemoglobin concentration than those with blood group B or A. However, there was no significant difference in parasite density between blood group O and A or B severe malaria patients. On the contrary, Migot *et al*. (2000) reported blood group O to be associated with low parasitemia (parasite density) than other blood groups.

The ABO determinants (blood group antigen A and B) are present on endothelial cells and platelets as well as erythrocytes, and these molecules are suggested to be receptors

for rosetting and sequestration (Cserti and Dzik, 2007). However, blood group antigen A and B are not expressed in individuals with blood group O. Therefore, rosetting and sequestration are lower in individuals with blood group O than the other blood groups. Since rosette formation was suggested to be associated with severity of the disease, i.e. severe anemia (Rowe *et al.*, 1995), the higher hemoglobin concentration in blood group O individuals may be due to the lower rosetting in blood group O individuals. Higher sequestration in blood group A and B due to the role of blood group antigen A and B may be the reason for lack of difference in parasite density between blood group O and A or B severe malaria patients.

In the present study, there was higher percentage of blood group A (35.7 %) than group O (22.9%), B (21%) and AB (20%). Furthermore, there were lower percentage of blood group O individuals (22.9%) in the severe malaria category than in the uncomplicated malaria (45.7%) and healthy control (41.6%) categories. This was consistent with the study in Sri Lanka (Pathirana *et al*., 2005), where there was low percentage of blood group O (24%) individuals as compared to the uncomplicated malaria (48%) and healthy controls (49%) and higher percentage of blood group A (33%) severe malaria patients. However, lack of previous similar studies poses difficulty in making rigorous discussion on percentage distribution of ABO blood group types in severe malaria patients in Ethiopia.

Along slide blood film examination (Giemsa stain microscopy), each blood sample was simultaneously tested for malaria parasite with Paramchek Pf (a rapid test for the detection of *P.falciparum)*. The false negative readings by Paramchek Pf were identified as *P.falciparum* under microscopy. This could be due to the insufficient antigenaemia during early malaria infection as shown by Juntra *et al*. (1996). The antigen HRP-2 is derived from trophozoite, and it is possible that during early infection the trophozoite may not have expressed enough HRP-2 to be detected by the test.

This may suggest that there is no correlation between the positivity of the test and the parasitaemia, which is mainly due to the small ring forms, not trophozoite.

6. CONCLUSION AND RECOMMENDATION

CONCLUSIONS

1. This study suggested that ABO blood group of the human host can influence the severity of disease due to infection with *P. falciparum* malaria.
2. Cases of severe malaria were less likely to be of blood group O, and more likely to be of blood group AB than the patients with uncomplicated malaria.
3. Blood group O doesn't protect against severe malaria by reducing parasite burden.

RECOMMENDATIONS

1. Since ABO blood group can also affect the severity of *P. falciparum* malaria by mechanisms other than rosetting, further studies of rosette formation with Ethiopian strains of *P. falciparum* could be useful to test the association between rosette formation and ABO blood group types.
2. Since sequestration of *P. falciparum* infected *RBC* in capillaries results in an underestimate of parasite count, in addition to parasitemia there must be other methods that will determine parasite load by accounting for the effect of sequestration.
3. Studies that will investigate the relationship between ABO blood group and malaria should focus on both clinical as well as laboratory markers of severe malaria.

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8. APPENDICES

Annex I: Socio-demographic data collection format

Health institution _____

Name _____

Code no._____

Age _____

Sex_____

Address _____

Length of residence in the area_____

Have you seen any symptoms? 1. Yes 2. No

If yes, what are the symptoms? _____

Have you had drug treatment for malaria before 48 hours? 1. Yes 2. No

Responsible person_____ Signature _____

Annex II: Blood collection format

Patient Id _____

Sample Id _____

Malaria examination result / Microscopy 1.Positive 1.1 P/f 1.2 P/v

2. Negative

Malaria examination result/ RDT 1.Positive

2. Negative

Annex III: Hemoglobin measurement format

Patient Id _____

Sample code _____

Hemoglobin results _____mg/dl

DECLERATION

I, the undersigned, declare that this thesis is my own original work, has not been presented for a degree in any University and that all sources of materials used for this thesis have been dully acknowledged.

Name: Zinaye Tekeste

Date _____

Signature_____