ASSOCIATION OF ABO BLOOD GROUPS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX INFECTION AND SEVERITY OF THE CLINICAL STATE AT ARBA MINCH GENERAL HOSPITAL, GAMO GOFA, ETHIOPIA.

A thesis submitted to Addis Ababa University, College of Health Sciences, School of Medicine, Department of Medical Physiology in partial fulfillment of the requirement for the Degree of Master of Science in Medical Physiology.

By: Gizachew Girma Cholje (BSc.)

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Addis Ababa, Ethiopia
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Declaration

This research is my original work and has not been presented for any degree in any other university, and all sources of materials used were appropriately acknowledged.

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TABLE OF CONTENTS

ACKNOWLEDGMENT ........................................................................................................................ I

LIST OF TABLES ................................................................................................................................ VI

LIST OF FIGURES ............................................................................................................................... VII

LIST OF ACRONYMS ........................................................................................................................ VIII

ABSTRACT ........................................................................................................................................ IX

Chapter One: Introduction ..................................................................................................................... 1

1.1 Background .................................................................................................................................. 1

1.2 Literature Review ............................................................................................................................ 4

  1.2.1 Burden of malaria .................................................................................................................. 4

  1.2.2 Pathogenesis of malaria ........................................................................................................ 5

  1.2.3 Severe malaria ...................................................................................................................... 7

  1.2.4 ABO antigens ....................................................................................................................... 8

  1.2.5 ABO association with malaria infection and clinical severity of the disease ......................... 9

1.3 Statement of the Problem ............................................................................................................ 12

1.4 Significance of the Study ............................................................................................................. 14

Chapter Two: Objectives ...................................................................................................................... 15

2.1 General Objective ......................................................................................................................... 15

2.2 Specific Objectives ....................................................................................................................... 15
Chapter Three: Materials and Methods .......................................................... 16

3.1 Materials .................................................................................................. 16

3.1.1 Study area ......................................................................................... 16

3.1.2 Study period ..................................................................................... 16

3.1.3 Source population ........................................................................... 16

3.1.4 Study population ............................................................................. 16

3.1.5 Study equipments ........................................................................... 17

3.2 Methods ................................................................................................ 17

3.2.1 Study design .................................................................................... 17

3.2.2 Sample size determination ............................................................... 17

3.2.3 Inclusion and exclusion criteria ...................................................... 19

3.2.4 Sampling procedure ....................................................................... 19

3.2.5 Study variables ............................................................................... 19

3.2.6 Specimen collection and preparation ............................................. 20

3.2.7 Data collection procedures .............................................................. 20

3.2.8 Laboratory analysis ....................................................................... 20

3.2.9 Physical examination ..................................................................... 21

3.2.10 Statistical analysis ......................................................................... 22

3.2.11 Data quality assurance ................................................................. 23
LIST OF TABLES

Table 1. Gender of the study participants.................................................................24
Table 2. Frequency distribution of plasmodium species among male and female respondents… 28
Table 3. Frequency distribution of identified plasmodium species among age groups of the study participants...........................................................28
Table 4. Frequency distribution of parasite density among the four blood groups.............30
Table 5. Types of complicated malaria.....................................................................31
Table 6. Frequency distribution of plasmodium species in the two malaria categories........33
Table 7. Frequency distribution of plasmodium species among the four blood groups........35
Table 8. Frequency distribution of parasite density among the two malaria categories........36
Table 9. Frequency distribution of blood group A and non-A among two malaria categories... 37
Table 10. Odds ratio between blood group A and other blood groups for clinical state of malaria ........................................................38
Table 11. Frequency distribution of blood group B and non-B among two malaria categories.... 38
Table 12. Frequency distribution of blood group O and non-O among two malaria categories.... 39
Table 13. Odds ratio between blood group O and other blood groups for clinical state of malaria .................................................................40
Table 14. Frequency distribution of ABO blood groups among the two categories of malaria ...... 42
LIST OF FIGURES

Figure 1. Frequency distribution of gender among age category of the study participants……25
Figure 2. Frequency distribution of ethnicity of the respondents…………………………26
Figure 3. Educational status of the study participants……………………………………..27
Figure 4. Frequency distribution of the ABO blood groups among study participants……32
Figure 5. Frequency distribution of ABO blood groups among uncomplicated and complicated malaria …………………………………………………………………………………..41
### LIST OF ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CD36</td>
<td>Cluster determinant 36</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CR-1</td>
<td>Complement receptor 1</td>
</tr>
<tr>
<td>CLD</td>
<td>Chronic liver disease</td>
</tr>
<tr>
<td>CM</td>
<td>Cerebral malaria</td>
</tr>
<tr>
<td>CSA</td>
<td>Chondroitin sulphate A</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>HMIS</td>
<td>Health Management Information System</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intracellular adhesion molecule 1</td>
</tr>
<tr>
<td>IE</td>
<td>Infected erythrocytes</td>
</tr>
<tr>
<td>iRBCs</td>
<td>Infected red blood cells</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PfEMP1</td>
<td><em>Plasmodium falciparum</em> erythrocyte membrane protein 1</td>
</tr>
<tr>
<td>RD</td>
<td>Respiratory distress</td>
</tr>
<tr>
<td>SA</td>
<td>Severe malarial anemia</td>
</tr>
<tr>
<td>SM</td>
<td>Severe Malaria</td>
</tr>
<tr>
<td>SNNPR</td>
<td>Southern Nation, Nationalities and Peoples Region</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule</td>
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ABSTRACT

**Background:** Malaria is an infection caused by protozoan parasites of the genus plasmodium. A broad range of available evidences suggest that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *P. falciparum* infection. ABO antigens are oligosaccharides that are attached to the proteins and lipids on the surface of RBCs and they can regulate protein activities during infection.

**Objective:** To assess an association of ABO blood groups with *Plasmodium falciparum* and *Plasmodium vivax* infection and severity of the clinical state at Arba Minch General Hospital, Gamo Gofa, Ethiopia 2017 G.C.

**Method:** Institution based cross-sectional study was used at Arba Minch General Hospital from September to November 2017 G.C. Data were analyzed using SPSS software (version 21.0). Chi-square ($X^2$) and odds ratio (OR) were used to determine the association between ABO blood groups and malaria infection and severity of the clinical state. Values were considered statistically significant when P-value is less than 0.05. Sample size was determined by using single population proportion formula with proportion (p) taken as 0.173 at 95% confidence interval and margin of error (5%).

**Results:** In the severe malaria category, there were 56.9%, 21.6%, and 21.6% blood group A, B and O patients respectively. A case of severe (complicated) malaria was almost three times as likely to be of type A as to be of type O (OR=2.84, 95% CI=1.31-6.17, P=0.008) and nearly twice as likely to be of type B as to be of type O (OR=1.72, 95% CI=0.68-4.28, P=0.026). None of the individuals with blood group AB developed severe (complicated) malaria.
Conclusion: This study revealed that individuals with blood group A have increased risk of developing severe (complicated) malaria than individuals with other blood groups, whereas individuals with blood group O have significantly less risk of developing severe (complicated) malaria than individuals with other blood group phenotypes.

Key words: ABO Blood groups, P.falciparim, P.vivax, severe (complicated) malaria.
Chapter One: Introduction

1.1 Background

Malaria is an infection caused by protozoan parasites of the genus plasmodium and transmitted by the bite of infected Anopheles mosquitoes. Out of the four species that infect humans, *Plasmodium falciparum* is the principal cause of severe clinical disease (Tekeste & Petros, 2010).

Malaria is one of the most severe public health problems and a leading cause of death in many developing countries especially in Africa (Simon-Oke, Afolabi, & Itansanmi, 2016). In Ethiopia, more than 75% of the total area is malarious, and malaria is also the major public health problem in Ethiopia (Getnet Ayele, Zewotir, & Mwambi, 2012; FDRE/MoH, 2004).

The number of malaria cases is estimated to have fallen from 262 million globally in 2000 (range 205–316 million) to 214 million in 2015 (range 149–303 million), a decline of 18%. The number of malaria deaths fell from an estimated 839,000 globally in 2000 (range 653,000 to 1.1 million), to 438,000 in 2015 (range 236,000–635,000), a decline of 48%. Most cases (88%) and deaths (90%) in 2015 were estimated to have occurred in Africa (“World Malaria Report,” 2015).

At the beginning of 2016, malaria was considered to be endemic in 91 countries and territories. Despite this remarkable progress, malaria continues to have a devastating impact on people’s health and livelihoods. Updated estimates indicate that 212 million cases occurred globally in 2015, leading to 429,000 deaths, most of which were in children aged under 5 years in Africa (World Malaria Report, 2016).
Therefore, the burden is heaviest in Africa, especially sub-Saharan countries where the disease is most severe and affects the poorest communities that have very limited access to services of prevention, diagnostics, and treatment (Palmkvist, 2016).

A broad range of available evidences suggest that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *P. falciparum* infection (Yibro Hussen, 2014). Clinical reports of ABO blood groups and *P. falciparum* infection, reveals a correlation between disease severity and ABO blood group (Deepa Ross, Alwar, & Rameshkumar, 2011).

*P. falciparum* infection is linked to the most severe forms of human malaria and virulence is associated with parasite reproduction rate and erythrocyte invasion mechanism (Chotivanich et al., 2000). Cytoadherence (binding of infected red blood cells to micro vascular endothelial cells in various organs) and rosetting are important components of several possible pathogenic mechanisms attributed to the cause of severe infection (Ringwald et al., 1993).

Trisaccharides of ‘A’ and ‘B’ blood group are presumed to act as receptors and function as an important factors for rosetting (Barragan, Kremsner, Wahlgren, & Carlson, 2000). However, RBCs of blood group ‘O’ do not express trisaccharides, and rosettes formed by infected ‘O’ blood group RBCs are smaller and easily disrupted when compared to blood groups A, B or AB (Carlson & Wahlgren, 1992).

An association between ‘O’ blood group and lower rosetting capacity has been demonstrated (Uneke, 2007). In addition, rosetting was significantly associated with severe malaria in patients with blood groups A, B and AB. However, this relationship was not apparent in patients with blood group O (Rowe et al., 2007).
Several studies have been done since the discovery of ABO antigens, to find out an association between ABO blood group antigens and susceptibility, resistance, or severity of *P. falciparum* malaria. Research done in northwestern Ethiopia indicated that severe malaria occurs more frequently in blood group A than other blood groups (Tadesse & Tadesse, 2013).

It is also reported that blood type A is the most often affected by severe malaria and rosettes formed in blood types A and B are larger, tighter and stronger than those formed in persons with blood type O (Qijun, Schlichtherle, & Wahlgren, 2000). However, some other researchers investigated that the risk of developing severe malaria is higher among blood group AB than other blood groups (Fry et al., 2008; Pathirana et al., 2005; Tekeste & Petros, 2010).

Several investigators reported that blood group O has a protective effect against severe *P. falciparum* malaria than other blood group phenotypes (Panda et al., 2011; Rowe et al., 2007; Fry et al., 2008; Tekeste & Petros, 2010). However, the effect of blood group O against *P. falciparum* infection and parasite replication was not significant (Rowe et al., 2007).

However, few studies reported that there was no significant association between ABO blood groups and prevalence of malaria (Montoya et al., 1994; Uneke, 2007). Therefore, literatures regarding the association between ABO blood groups and malaria infection as well as severity of the disease were not clearly elucidated the association.
1.2 Literature Review

1.2.1 Burden of malaria

Malaria is an infectious disease caused by a one-celled obligate intra-erythrocytic protozoan of the genus plasmodium. *P. falciparum* is responsible for the most severe form of the disease and accounts for over 96% of all malarial infections in sub-Saharan Africa (Minja, 2013). Malaria is one of the most severe public health problems and a leading cause of death in many developing countries especially in Africa (Simon-Oke et al., 2016). More than 75% of the total area of Ethiopia is malarious, and it is also a major public health problem in Ethiopia (Getnet Ayele, Zewotir, & Mwambi, 2012; FDRE/MoH, 2004).

Globally 3.3 billion people are at risk of malaria infection. Eighty percent of the 219 million malaria cases in 2010 and 90% of 660,000 malaria-related deaths were from Africa (WHO Global Malaria Programme, 2012). In 2013, about 584,000 people globally died from malaria; nearly 90% of the deaths occurred in Sub-Saharan Africa where *P. falciparum* is the most prevalent of the malaria parasites and the leading cause of malaria deaths (WHO, Severe Malaria, 2014).

At the beginning of 2016, malaria was considered to be endemic in 91 countries and territories. Despite this remarkable progress, malaria continues to have a devastating impact on people’s health and livelihoods. Updated estimates indicate that 212 million cases occurred globally in 2015, leading to 429,000 deaths, most of which were in children aged under 5 years in Africa (World malaria report, 2016).
The problem of malaria is also very severe in Ethiopia where it has been the major cause of illness and death for many years. According to records from the Ethiopian Federal Ministry of Health, 75% of the country is malarious with about 68% of the total population living in areas at risk of malaria. That is, more than 50 million people are at risk from malaria, and four to five million people are affected by malaria annually (Getnet Ayele, Zewotir, & Mwambi, 2013).

1.2.2 Pathogenesis of malaria

During development in the red blood cell, the parasite transports and expresses polypeptides on the cell surface making the membrane of the infected erythrocyte "sticky". These sticky proteins mediate adhesion to uninfected red blood cells forming, so-called "rosettes". Moreover, these proteins can also bind to endothelial cells in the microvasculature, a phenomenon known as “sequestration”, which leads to severe obstruction of the blood flow in the host (Palmkvist, 2016).

Erythrocyte rosetting (binding of one infected RBC to two or more uninfected RBCs) is linked to the pathogenesis of severe malaria phenotypes such as cerebral malaria (CM) and severe malarial anemia (SA). *P.falciparum* erythrocyte membrane protein 1 (PfEMP1) has been identified as the rosetting ligand of the parasite (Fry et al., 2008).

The PfEMP1s are not expressed on early ring stage parasites, which still have the ability to pass through the spleen, but emerges on the IE surface as the parasite develops into the late ring stage (early trophozoite).

When expressed on the erythrocyte surface, the PfEMP1s are found on small protrusions, so-called knobs, which are thought to increase the adhesion of the IE (Vang Petersen, 2016).
Trisaccharides of ‘A’ and ‘B’ blood group are presumed to act as receptors and function as an important factor for rosetting (Barragan et al., 2000). Furthermore, blood group antigens A and B can also act as co-receptors in rosette formation, and isolates show different rosetting rates and rosette sizes when cultured with erythrocytes of different blood groups. Rosette formation may also involve complement receptor1 (CR1) on the surface of RBCs. Red cells deficient in CR1 do not form rosettes, and soluble CR1 or antibodies can inhibit rosette formation in a range of isolates (Beeson & Brown, 2002).

In addition to rosetting, cytoadherence (binding of infected red blood cells to microvascular endothelial cells in various organs) has a crucial role in the pathogenesis of severe malaria. *P. falciparum* infected red blood cells (iRBCs) bind to host endothelial cells and other RBCs which physically block the blood vessels leading to vascular occlusion. On a molecular level, cytoadherence is caused by the export of parasite proteins to the RBC surface which then interact with receptors on the surface of host cells such as CD36, ICAM-1, VCAM, CSA and others (Sedillo, 2014).

Rosetting has been established as a *P. falciparum* virulent factor, the expression of which is modified by a variety of host factors which include the ABO and Rh blood types (Christine M. Cserti & Walter H. Dzik, 2007). Therefore, rosetting and sequestration are considered as major pathological features of severe malaria (Palmkvist, 2016).
1.2.3 Severe malaria

In a patient, a *P. falciparum* infection with asexual parasitaemia and no other confirmed cause for their symptoms or signs, the presence of one or more of the following clinical manifestations (impaired consciousness, respiratory distress (acidotic breathing), multiple convulsions, prostration, shock (systolic blood pressure <50mmHg in children and <80mmHg in adults with impaired perfusion (cool peripheries or prolonged capillary refill)), pulmonary edema (radiological), abnormal bleeding and jaundice) or laboratory features (severe anemia (a hemoglobin concentration <5g/dl or hematocrit of <15% in children <12 years of age (<7g/dl or <20% respectively in adults)), hypoglycemia (blood or plasma glucose < 2.2mM or <40mg/dl), acidosis, hyperlactatemia, renal impairment and hyperparasitaemia) classifies that patient as suffering from severe malaria (WHO, Severe Malaria, 2014).

*P. falciparum* infections are linked to the most severe forms of human malaria and virulence is associated with parasite reproduction rate and erythrocyte invasion mechanism (Chotivanich et al., 2000). *P. falciparum* causes the most deaths of the five plasmodium species due to its ability to cause a severe form of malaria. The severity is due to its rapid growth cycle and ability to cytoadhere. *P. falciparum* cases have the highest average parasitemia rate in the blood (10-100x that of the other species) (Vang Petersen, 2016).

Clinical manifestations of severe *P. falciparum* infection are variable in nature and severity, and it can be difficult to clearly define syndromes in many cases. A cerebral malaria syndrome appears to account for the majority of malaria deaths.
A diagnosis of cerebral malaria requires definite evidence of malaria infection; asexual forms of *P. falciparum* in the blood film and a Glasgow Coma Score of less than 11 and ‘Unrrousable coma’ which is defined as a best motor response to noxious stimuli that is ‘non localising’, and a best vocal response that is considered ‘incomprehensible’ often with convulsion.

Severe normocytic anemia is probably the second most common presentation of severe *P. falciparum* infection and probably results from increased RBC destruction and reduced erythropoiesis (Beeson & Brown, 2002). The most apparent complications found in severe malaria patients are malarial anemia, respiratory distress (RD), cerebral malaria (CM), and/or pregnancy-associated malaria (Vang Petersen, 2016).

### 1.2.4 ABO antigens

ABO antigens were discovered in 1900 by Austrian scientist and Nobel Laureate Karl Landsteiner, these antigens are oligosaccharides that are attached to the proteins and lipids on the surface of RBCs. These are A and B antigens which are trisaccharides attached to a variety of glycolipids and glycoproteins on the membrane surface of red blood cells.

Blood group O individuals lack the enzyme glycosyltransferase necessary to produce the A or B antigens and carry the disaccharide H antigen (Daniels, 2005). These antigens can regulate protein activities during infection and antibodies against them (Gayathri, Harendra, M.L, & Jeevan Shetty, 2013).

In clinical practice, ABO system is the most important system for blood group compatibility. In the century since their discovery, ABO antigen associations with infections and other diseases have been the subject of hundreds of publications.
Some reports found unexpected associations, such as the susceptibility of blood group A individuals to salivary or gastric cancer. Much new information has emerged since a relationship between ABO antigens and malaria was first suggested more than 40 years ago (Christine M. Cserti & Walter H. Dzik, 2007).

### 1.2.5 ABO association with malaria infection and clinical severity of the disease

Since the discovery of the ABO blood groups, numerous associations between ABO groups and various diseases have been noted (Christine M. Cserti & Walter H. Dzik, 2007). Various investigators have searched to establish an association between the ABO blood types and malaria infection and severity of clinical state.

A study conducted in Kenya showed that the highest proportion of malaria infection was observed among respondents with blood group A followed by those with blood group O. Malaria infection was significantly associated with blood group A. Blood group O was not significantly associated with getting malaria infection (Simiyu, 2013).

In another study, malaria infection showed significant association with blood groups with the highest proportion among individuals with AB blood group, followed by those with blood group B and the chance of having *P. falciparum* infection was less in blood type O than other blood groups (Zerihun, Degarege, & Erko, 2011).

Similarly, others reported that blood group AB was significantly infected with malaria parasites than other blood groups (Simon-Oke et al., 2016). However, in other study maximum numbers of malaria infection was observed in blood group ‘O’ positive patients than other blood group patients (Singh et al., 2015).
A study carried out with children in age between 0-24 months in Nigeria, showed that the frequency of malaria infection was highest among children with blood group A than others (Onanuga and Lamikanra, 2016).

Another study done in south India, reported that individuals with blood group A and B are more susceptible to malaria infection as compared with individuals of blood group O (Gayathri et al., 2013).

However, other researchers reported contradicting results with the one mentioned above that the highest malaria rate of infection was observed among individuals with blood group O and the lowest was seen among blood group B individuals (Nkou-Akenji, Paul, & Akoachere, 2004).

A study conducted in Odisha state India, reported that, from 247 patients with severe malaria, patients with blood group ‘B’ have a four-fold increased risk of developing severe infection but patients with ‘O’ blood group have significantly decreased risk of developing severe malaria and other blood groups (‘A’ and ‘AB’) did not show any association (Panda et al., 2011).

Similarly, among Malian children blood group “O” confers significant protection against severe malaria compared with the non-“O” blood groups and has no protective effect against uncomplicated malaria.

Therefore, blood group O only protects against severe, life-threatening malaria and not against uncomplicated clinical malaria with low or high parasite burdens (Rowe et al., 2007). Other researchers also reported that the protective effect of blood group O was demonstrated with a decreased risk of severe malaria (Amodu et al., 2012).
In northwest Ethiopia, severe malaria infection was significantly associated with non ‘O’ blood groups with the highest proportion of severe disease among participants with blood group A and least proportion among O blood group (Tadesse & Tadesse, 2013).

Another large-scale study conducted in Gambia, Kenya and Malawi indicates that blood group A, B and AB individuals appear to be at significantly greater risk of developing severe malaria in comparison with blood group O. In the same study blood group B individuals may be at subtly lower risk than blood group A, while blood group AB individuals are probably at the greatest risk of severe disease (Fry et al., 2008).

A study done in Sri Lanka showed that patients with severe malaria were less likely to be of type O and more likely to be A, B or AB when compared with patients with uncomplicated malaria. In terms of odds ratio, a case of severe malaria was almost three times as likely to be of type A as to be of type O, and more than twice as likely to be of type B as to be of type O, and patients with type AB were about seven-fold more likely to develop severe disease than type O patients (Pathirana, Alles, & Bandara, 2005).

Similarly a study done in malaria-endemic regions of Ethiopia including Awash, Metehara and Ziway areas, indicated that a case of severe malaria was almost twice as likely to be of type A as to be of type O, and more than twice as likely to be of type B as to be of type O. Furthermore, individuals with severe malaria were about six-fold less likely to be of O as to be of type AB (Tekeste & Petros, 2010).
1.3 Statement of the Problem

Malaria is one of the leading causes of mortality and morbidity in Ethiopia, from mid-2013 until mid-2014; HMIS reported a total of 2,383,010 malaria illnesses including 1,256,611 outpatient *P. falciparum* malaria illnesses; 16,326 malaria admissions from *P. falciparum*; and fewer than 450 malaria deaths (“President’s Malaria Initiative Ethiopia Malaria Operational Plan FY 2016,” 2016).

Even though the proportion of malaria varies from place to place and season to season, *P. falciparum* and *P. vivax* are the two dominant parasite species with a relative frequency of 60% and 40%, respectively in Ethiopia (FDRE/MoH, 2004). In malaria epidemic situations, *P. falciparum* is the dominant species that causes major clinical manifestations and almost all malaria deaths (Yibro Hussen, 2014).

Many communities in the country have a “low” and “unstable” malaria transmission pattern that results in low host immunity and significant clinical disease risk for malaria after malaria infections, increased tendency for rapid progression to severe malaria, and propensity for malaria epidemics affecting all age groups (“President’s Malaria Initiative Ethiopia Malaria Operational Plan FY 2016,” 2016).

From time to time, it has been discovered that there are certain individuals who are more susceptible to *P. falciparum* malaria than others living in the same endemic regions. This could be accounted for by several factors including developing an immune response by the host, sickle cell trait, hemoglobin variants, ABO blood groups and the level of G-6-P-D enzyme action (Otajevwo, 2013).
The association of genetic markers with malaria has been the subject of numerous investigations since the finding show protection afforded by sickle-cell hemoglobin against infection by *P. falciparum* malaria parasite (Deepa Ross et al., 2011). Clinical reports of ABO blood groups and *P. falciparum* infection, reveals a correlation between disease severity and ABO blood groups (Deepa Ross et al., 2011; Fry et al., 2008; Pathirana et al., 2005; Tekeste & Petros, 2010).

There is increasing evidence that *P. falciparum* malaria is influenced by ABO blood groups, however, the extent of association is yet to be well defined. There were apparent discrepancies and contradictions in the studies about the relation between ABO blood groups and *P. falciparum* as some reported significant association between ABO blood groups and *P. falciparum* while others observed no significant association between them (Uneke, 2007). In addition, several studies undertaken have been unable to show a link between ABO blood groups to the incidence of malaria or to the repeat attacks of malaria (Singh, Urhekar, & Singh, 2015a).

Therefore, an investigation of malaria in relation to ABO blood group antigens has the potential of giving an insight into the pathogenesis of malaria in patients with different blood groups and perhaps help in the prevention and control of the disease.
1.4 Significance of the Study

The association of ABO blood groups and *P. falciparum* malaria has been demonstrated in various populations. Arba Minch town and the rural surroundings are one of the most malaria endemic areas of southern Ethiopia, where malaria has most public health importance. However, there is no study that tested the association between ABO blood groups and the severity of the clinical state of malaria caused by *P. falciparum* and/or *P. vivax* in Gamo Gofa, Arba Minch area. Knowing an association between ABO blood groups and malaria infection and severity of the clinical state will help health care providers to predict the disease progress in terms of severity as well as to treat patients carefully. Therefore this study was conducted to investigate the association between ABO blood groups and *P. falciparum* and *P. vivax* infection and severity of the clinical state of the disease in the study area.
Chapter Two: Objectives

2.1 General Objective

To assess the association of ABO blood groups with *Plasmodium falciparum* and *Plasmodium vivax* infection and severity of the clinical state at Arba Minch General Hospital, Gamo Gofa, Southern Ethiopia.

2.2 Specific Objectives

1. To assess the frequency distribution of ABO blood groups among patients with *Plasmodium falciparum* and *Plasmodium vivax* infection.

2. To describe the association of ABO blood groups with *Plasmodium falciparum* and *Plasmodium vivax* infection.

3. To determine the association of ABO blood groups with severity of the clinical state of malaria infection caused by *Plasmodium falciparum* and *Plasmodium vivax*. 
Chapter Three: Materials and Methods

3.1 Materials

3.1.1 Study area

The study was conducted at Arba Minch General Hospital. Arba Minch town is located at about 462 km southwest of the capital, Addis Ababa. The area is one of the malaria endemic areas in the southern region of Ethiopia with an elevation ranging from 1200 to 1300m above sea level; annual temperature ranging from 28°C–43°C and annual rainfall of about 818mm. The estimated total population of the study area was about 113,297 of which 55,516 are males and 57,781 are females.

3.1.2 Study period

The study was conducted from September to November 2017 G.C.

3.1.3 Source population

All malaria suspected patients with acute febrile illness attending Arba Minch General Hospital during the study period.

3.1.4 Study population

Malaria confirmed (thin and thick blood film) cases at Arba Minch General Hospital during the study period and fulfilling the inclusion criteria were included.
3.1.5 Study equipments

- WHO standardized questionnaire (Amharic version) was used to assess the socio-demographic characteristics of participants.
- Aneroid sphygmomanometer was used to measure blood pressure.
- Thick and thin blood film slides were prepared using 10% Giemsa solution.
- Light microscope with 100 × oil immersion was used to identify plasmodium species and determine parasite count.
- Commercial antisera for RBC antigens A, B and D were used for agglutination.
- Disposable gloves, syringe (5cc) with needle, test tubes and capillary tubes were used to collect and prepare specimen.
- Haemoglobinometer was used to determine hemoglobin concentration.
- Glucometer was used to analyze a tiny drop of blood for blood glucose level.

3.2 Methods

3.2.1 Study design

Institution-based cross-sectional study was employed.

3.2.2 Sample size determination

Sample size was determined by using single population proportion formula based on the following assumptions: In EFY 2007, the number of laboratory confirmed plus clinical malaria cases was 375,746 in SNNPR and the number of population at risk in the same region was 12,238,426 with malaria incidence (17.3%) (FDRE/MOH, 2015).
To determine the required sample size, the proportion (p) was taken as 0.173 at 95% confidence level and margin of error 5%

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

$$n = \left( \frac{Z_{\alpha/2}}{d} \right)^2 \cdot p \cdot q$$

Where:

- n = sample size
- p = sample proportion
- q = 1 - p
- d = margin of error (5%).
- \(Z = \) is the standard normal value at the level of confidence desired at 95% CI.

Therefore:-

\(Z_{\alpha/2}\)-value will be 1.96, at the standard normal value of 5% level of significance

P-value will be 0.173 since currently the prevalence of malaria at SNNPR is 17.3% (FDRE/MOH, 2015)

d= margin of error will be 0.05

Sample Size (n) = \((1.96 \times 1.96 \times 0.173 \times 0.827)\)/(0.05 \times 0.05)

=219.84~220

Expecting a 5% non-response rate, the final sample size calculated was 231.
3.2.3 Inclusion and exclusion criteria

3.2.3.1 Inclusion criteria

Patients who were suspected to have malaria and confirmed positive for malaria parasites (*P.falciparum*, *P.vivax* or both) using thick and thin blood smear by microscopy were included.

3.2.3.2 Exclusion criteria

*P. falciparum* or *P. vivax* positive individuals who had received anti-malarial treatment (drugs) within 48 hours prior to the microscopical confirmation, those co-infected with *P. falciparum* and other non-*P. vivax* species or *P.vivax* and other non-*P. falciparum* species, children under five year, pregnant women, individuals with immune suppressive diseases (HIV-AIDS, DM, CLD) and those above 60 years were excluded from the study.

3.2.4 Sampling procedure

The study subjects were selected by using purposive sampling to sequentially enroll patients diagnosed with malaria until the required sample size is reached.

3.2.5 Study variables

3.2.5.1 Dependent variables

Malaria infection, clinical state of malaria

3.2.5.2 Independent variables

Age, sex, ethnicity, blood group, parasite density
3.2.6 Specimen collection and preparation

The required amount of specimen was collected and prepared by trained health professionals (medical laboratory technologists) before determination of blood group, parasite density, hemoglobin concentration and random blood sugar.

3.2.7 Data collection procedures

Socio-demographic characteristics and other required information were collected from the participants by face to face interview using Amharic version of the structured questionnaire by nurses. About 5ml of venous blood and capillary blood was collected by qualified health care professionals (medical laboratory technologists) in the Hospital.

3.2.8 Laboratory analysis

3.2.8.1 Parasite density determination

Thick and thin blood film slides were prepared using 10% Giemsa solution. The slides were stained and examined under a light microscope using 100 × oil immersions by an experienced laboratory technician. Parasite density was calculated per 200 white blood cells (WBC) assuming 8000 WBC/μl of blood (Tekeste & Petros, 2010).

3.2.8.2 Blood group determination

ABO blood groups were typed by agglutination test using commercial antisera (Biotech laboratories Ltd, Ipswich, Suffolk, UK). Two drops of whole blood were placed in three different places of a grease-free clean glass slide on which a few drops of antisera for blood group A and B as well as D antigen were applied.
The blood cells and the antisera were mixed with an applicator stick. Then the slides were tilted to detect for agglutination and the result was recorded accordingly (Tekeste & Petros, 2010).

### 3.2.8.3 Determination of hemoglobin concentration

Finger-prick samples were collected and hemoglobin concentration was measured by a device called Hemocue™ (haemoglobinometer, Angelholm, Sweden).

### 3.2.8.4 Determination of random blood sugar

Random blood sugar was determined by using a glucometer which rapidly analyzes a tiny drop of blood and then displays blood glucose level at that moment. To obtain a blood sample, a spring-loaded device that pricks the skin with a small pointed piece of surgical steel called a lancet was used.

### 3.2.9 Physical examination

#### 3.2.9.1 Blood pressure measurement

Blood pressure was measured by using a device called mercury sphygmomanometer. During measurement, the participants sat on a chair with back supported, legs uncrossed and feet on the floor. Their arm turn outward so their palms were facing upward and any heavy clothing over arms was removed then their arms were relaxed for at least five minutes. In addition, the participants’ arms were supported over table so that the location of upper arms at the level of heart was ensured. Then the measurement procedure was done and both systolic and diastolic blood pressures were recorded.
3.2.9.2 Determining Glasgow Coma Scale (GCS) score

Glasgow coma scale score was determined for participants with impaired consciousness by Physicians in the outpatient and emergency case team. The GCS uses three categories that pertain to different areas of a person’s conscious state, they are; eyes opening, vocal response and motor response. Each unit is given a range of numbers that correlate with definable levels in consciousness.

<table>
<thead>
<tr>
<th>Eyes Opening</th>
<th>Verbal Response</th>
<th>Motor Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Spontaneous</td>
<td>5 Orientated to time and place</td>
<td>6 Obey's command</td>
</tr>
<tr>
<td>3 Voice</td>
<td>4 Confused speech</td>
<td>5 Localizes to pain</td>
</tr>
<tr>
<td>2 Pain</td>
<td>3 Inappropriate words</td>
<td>4 Withdraws to pain</td>
</tr>
<tr>
<td>1 None</td>
<td>2 Incomprehensible sound</td>
<td>3 Decorticate position</td>
</tr>
<tr>
<td></td>
<td>1 None</td>
<td>2 Decerebrate position</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 None</td>
</tr>
</tbody>
</table>

3.2.10 Statistical analysis

Data were analyzed using SPSS software (version 21.0, Chicago, IL, USA). Chi-square ($X^2$) test was used to determine the association between ABO blood groups and malaria infection and severity of the clinical state. Odds ratios (OR) was calculated at 95% confidence interval (CI). Values were considered statistically significant when P-value was less than 0.05.
3.2.11 Data quality assurance

The socio-demographic characteristics and related data were collected by professional nurses and all the laboratory procedures were handled by laboratory technologists. Tools used to measure and analyze the variables were standardized and automated. The data were enrolled into EpiData version 3.1 and cleared and checked for correctness and completeness before analysis.

3.3 Ethical Clearance

The study protocol was reviewed and approved by Ethical Review Committee of the Department of Medical Physiology, Addis Ababa University. Study subjects participated in the study after explanation about the purpose of the study. Written informed consent was obtained from all study participants and mothers/caretakers of children under 18 years.

3.4 Dissemination and Utilization of Results

Hard and soft copies were submitted to the department of Medical Physiology. The findings from the study were presented to the department of Medical Physiology, College of Health Sciences, Addis Ababa University and other concerned and scientific bodies. Moreover, the study findings will be shared with concerned bodies at the zonal, regional and national level and submitted to a peer-reviewed journal for publication.
Chapter Four: Results

4.1 Socio-Demographic Characteristics of the Respondents

A total of 231 malaria-infected (laboratory confirmed) patients were involved in the study; among them, 51.1% were males and 48.9% were females (Table 1).

Table 1. Gender of the study participants

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>118</td>
<td>51.1</td>
</tr>
<tr>
<td>Female</td>
<td>113</td>
<td>48.9</td>
</tr>
<tr>
<td>Total</td>
<td>231</td>
<td>100</td>
</tr>
</tbody>
</table>

Regarding the age category of the study participants, about a quarter (24.2%) of the respondents were in the age range of 5-14 years and nearly half (46.3%) of the respondents were between 15-24 years where as more than a quarter (29.4%) were 25 and above (Fig 1). The mean age of the respondents was 21.2 years and the mean age of males (21.4 years) was nearly similar with that of females (20.9 years).
Figure 1. Frequency distribution of gender among age category of the study participants

More than half (53.2%) of the respondents were Ethiopian Orthodox church members and the remaining 43.7% and 3% were Protestants and Muslims, respectively. Majority of the respondents were from Gamo ethnic group and the others were from Gofa, Amhara and others (Fig 2).
Regarding the marital status of the respondents, most of them were single (71.9%), and the others were married (26.4%) and divorced (1.7%). The majority (61%) of the study participants were at the primary and secondary school level and about a quarter of them were employed; full time (20.3%) and part-time (6.9%) (Fig 3). The mean average monthly income of the study participants was about 840.91 Ethiopian birr.
4.2 Clinical and Laboratory Results

4.2.1 Frequency of identified plasmodium species

Out of the 231 malaria cases (serologically confirmed by microscopy), 140 were found to be infected with *P. falciparum*, 82 were infected with *P. vivax* and the remaining 9 were infected with both *P. falciparum* and *P. vivax* (mixed infection). The proportion of *P. falciparum* infection was higher in both males and females than the proportion of *P. vivax* and mixed infection but the observed difference was not statistically significant (P >0.05) (Table 2).
Table 2. Frequency distribution of plasmodium species among male and female respondents

<table>
<thead>
<tr>
<th>Gender</th>
<th>Plasmodium falciparum</th>
<th>Plasmodium vivax</th>
<th>Mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>71(60.2%)</td>
<td>42(35.6%)</td>
<td>5(4.2%)</td>
<td>118(51.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>69(61.1%)</td>
<td>40(35.4%)</td>
<td>4(3.5%)</td>
<td>113(48.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>140(60.6%)</td>
<td>82(35.5%)</td>
<td>9(3.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

$X^2$, P  

0.08, 0.96

$X^2$ (chi-square) P (p-value at 95% confidence interval)

Nearly half (46.3%) of the malaria-infected patients were in the age group ranging from fifteen to twenty-four years and the proportion of \textit{P. falciparum} was higher in all age groups. The observed difference between age groups and plasmodium species was not statistically significant (P >0.05) (Table 3).

Table 3. Frequency distribution of identified plasmodium species among age groups of the study participants

<table>
<thead>
<tr>
<th>Age group</th>
<th>Plasmodium falciparum</th>
<th>Plasmodium vivax</th>
<th>Mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-14</td>
<td>35(62.5%)</td>
<td>16(28.6%)</td>
<td>5(8.9%)</td>
<td>56(24.3%)</td>
</tr>
<tr>
<td>15-24</td>
<td>65(60.8%)</td>
<td>41(38.3%)</td>
<td>1</td>
<td>107(46.3%)</td>
</tr>
<tr>
<td>25-60</td>
<td>40(58.8%)</td>
<td>25(36.8%)</td>
<td>3</td>
<td>68(29.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>140(60.6%)</td>
<td>82(35.5%)</td>
<td>9(3.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

$X^2$, P  

7.19, 0.12

$X^2$ (chi-square) P (p-value at 95% confidence interval)
4.2.2 Clinical findings

To assess clinical severity of the disease, different clinical and laboratory findings were assessed. Clinically; level of consciousness, signs of respiratory distress (acidotic breathing), abnormal bleeding and yellow discoloration of the sclera (jaundice) were assessed. The level of consciousness was determined using Glasgow Coma Scale (GCS) score by Physicians in the case team.

Seventeen patients had impaired consciousness; fourteen of them had ‘unrousable coma’ which is defined as a best motor response to noxious stimuli that is ‘non-localizing’, and a best vocal response that is considered ‘incomprehensible’ often with convulsion. Two of the malaria cases had yellowish discoloration of sclera (jaundice) and none of the malaria-infected patients experienced respiratory distress and/or acidotic breathing and abnormal bleeding.

In addition to the above signs, blood pressure was measured for all malaria patients. Accordingly, about 92.2% of patients had normal systolic blood pressure ranging from 90 to 120 mmHg, 5.2% had hypotension (systolic blood pressure less than 90 mmHg) of which 9 had systolic blood pressure below 80 mmHg. The remaining (2.6%) had systolic blood pressure above 120 mmHg.

4.2.3 Laboratory (serological) findings

Serologically (laboratory examination) plasmodium species were identified by using both thick and thin blood film microscopically and parasite density was determined for all malaria-infected individuals.
The majority, (88.3%) of patients had low parasite density (10 slides with 50–200 parasites/μl and/or, 10 slides with 200–500 parasites/μl) and the case fraction (11.7%) had high parasite density (20 slides with 500–2000 parasites/μl).

About (15.2%) of individuals with blood group A and (7.8%) of individuals with blood group B had high parasite density. But the observed difference between blood groups with parasite density was not statistically significant (P >0.05) (Table 4).

Table 4. Frequency distribution of parasite density among the four blood groups

<table>
<thead>
<tr>
<th>Parasite density</th>
<th>Blood group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Low</td>
<td>78(84.8%)</td>
<td>47(92.2%)</td>
</tr>
<tr>
<td>High</td>
<td>14(15.2%)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>92(39.8%)</td>
<td>51(22.1%)</td>
</tr>
</tbody>
</table>

\(X^2, P\) 4.09, 0.66

Hemoglobin concentration and random blood sugar were measured to assess the extent of hemolytic anemia and hypoglycemia in relation to malaria infection. The mean hemoglobin concentration of the participants was about 12.15gm/dl and about 11.3% of the respondents were with hemoglobin concentration less than 7gm/dl.
The mean hemoglobin concentration was also calculated for the two malaria categories and it was less in complicated malaria patients (8.56gm/dl) than the uncomplicated malaria group (15.74gm/dl). Out of a total 231 malaria infected patients; only three were hypoglycemic (random blood sugar less than 40mg/dl).

Based on both clinical and laboratory findings, about 22.1% of the malaria patients were diagnosed as severe (complicated) malaria and the remaining 77.9% were diagnosed as uncomplicated malaria. Among the complicated malaria cases, severe malarial anemia was the commonest (41.2%) followed by cerebral malaria (25.5%) and algid malaria (shock) (15.7%) (Table 5).

Table 5. Types of complicated malaria

<table>
<thead>
<tr>
<th>Diagnosis of complicated malaria</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe malarial anemia</td>
<td>21</td>
<td>41.2</td>
</tr>
<tr>
<td>Cerebral malaria</td>
<td>13</td>
<td>25.5</td>
</tr>
<tr>
<td>Algid malaria (malaria with hemodynamic disturbance(shock))</td>
<td>8</td>
<td>15.7</td>
</tr>
<tr>
<td>Complicated malaria secondary to hypoglycemia</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>Severe malarial anemia with shock</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>Severe malarial anemia with jaundice</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td>Cerebral malaria with shock</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>
4.3 Frequency of ABO Blood Groups among the Study Participants

All patients with confirmed malaria infection were also tested for ABO blood groups. Accordingly; 39.8%, 22.1%, 3.9% and 34.2% were found to be blood types of A, B, AB, and O, respectively (Fig 4). Almost all (99.1%) of them were Rh positive.

The highest proportion of individuals in all blood groups were infected with *P. falciparum* as compared with other plasmodium species (*P. vivax* and mixed), and this difference was not statistically significant (P >0.05) (Table 7).

![Pie chart showing the frequency distribution of ABO blood groups among study participants.](image)

**Figure 4.** Frequency distribution of the ABO blood groups among study participants
Total of 231 malaria cases were classified as uncomplicated and complicated (severe) malaria, based on both clinical and laboratory results. About 22.1% cases were classified as complicated malaria (severe malaria) and the rest 77.9% were classified as uncomplicated malaria.

In uncomplicated malaria category, blood groups A, B, AB, and O were 35%, 22.2%, 5% and 37.8% respectively. Among the complicated malaria category, more than half (56.9%) had blood group A, and the remaining had blood group B (21.6%) and blood group O (21.6%). None of the individuals with blood group AB was classified as complicated malaria.

Furthermore, both categories of malaria cases were classified based on blood group and plasmodium species. More than half (53.3%) of uncomplicated malaria and most (86.3%) of the severe (complicated) malaria were caused by *P. falciparum* and the observed difference was statistically significant  \((P < 0.001)\) (Table 6).

Table 6. Frequency distribution of plasmodium species in two malaria categories

<table>
<thead>
<tr>
<th>Plasmodium species</th>
<th>Uncomplicated malaria</th>
<th>Complicated malaria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>96(53.3%)</td>
<td>44(86.3%)</td>
<td>140(60.6%)</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>79(43.9%)</td>
<td>3(5.9%)</td>
<td>82(35.5%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>5(2.8%)</td>
<td>4(7.8%)</td>
<td>9(3.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>180(77.9%)</td>
<td>51(22.1%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

\[X^2, P = 25.9, 0.001^*\]

\(X^2\) (chi-square)  \(P\) (p-value at 95% confidence interval) * (p-value significant)
When compared with uncomplicated malaria, the chance of getting complicated malaria among individuals infected with *P. falciparum* was significantly higher than those individuals with *P. vivax* infection (OR=12.06, 95% CI=3.61-40.34, P=0.001).

Similarly, the chance of getting complicated malaria among individuals with mixed infection was significantly higher than individuals with *P. vivax* infection (OR=21.06, 95% CI=3.66-121.06, P=0.001). Therefore, severe (complicated) malaria was significantly higher among individuals with *P. falciparum* and mixed infection than individuals with *P. vivax* infection (P < 0.001).

Nearly half (46.4%) of *P. falciparum* infected patients had blood group A, and the remaining 20.7%, 4.3% and 28.6% were blood groups B, AB and O, respectively. Among *P. vivax* infected individuals, about 43.9% were blood group O and the remaining 29.3%, 24.4% and 2.4% were blood group A, B and AB respectively.

The difference between plasmodium species (*P. falciparum* and *P. vivax*) in relation with ABO blood groups was not statistically significant (P > 0.05), that is malaria infection with either *P. falciparum* or *P. vivax* species was not associated with specific ABO blood groups (Table 7). Therefore, neither *P. falciparum* nor *P. vivax* parasite selectively infects specific blood groups from the four common blood group phenotypes.
Table 7. Frequency distribution of Plasmodium species among the four blood groups

<table>
<thead>
<tr>
<th>Plasmodium species</th>
<th>Blood group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>65(46.4%)</td>
<td>29(20.7%)</td>
</tr>
<tr>
<td>P. vivax</td>
<td>24(29.3%)</td>
<td>20(24.4%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>92(39.8%)</td>
<td>51(22.1%)</td>
</tr>
</tbody>
</table>

$X^2$, P               

$X^2$ (chi-square)    P (p-value at 95% confidence interval)  

9.49, 0.14

4.4 Parasite Density of Malaria-infected Patients

Parasite density (count) was determined for all malaria-infected individuals. The majority of them (88.3%) had low parasite density (10 slides with 50–200 parasites/μl and/or, 10 slides with 200–500 parasites/μl) and the remaining 11.7% had high parasite density (20 slides with 500–2000 parasites/μl).

About 95.6% of individuals with uncomplicated malaria had low parasite count and only 4.4% of them had high parasite density. In complicated malaria category, about 62.7% of them had low parasite density and 37.3% had high parasite count (Table 8). Therefore, parasite density was found to be significantly different (P < 0.001) among uncomplicated and complicated malaria categories.
As compared to uncomplicated malaria, the chance of getting complicated malaria was less in individuals with low parasite density (OR=0.078 at 95% CI=0.032-0.194, P=0.00) than those with high parasite density. Similarly individuals with high parasite density have nearly thirteen times more probability of having complicated malaria than those with low parasite density (OR=12.76 at 95% CI=5.14-31.65, P=0.00).

Table 8. Frequency distribution of parasite density among the two malaria categories

<table>
<thead>
<tr>
<th>Parasite density</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>19(37.3%)</td>
<td>8(4.4%)</td>
<td>27 (11.7%)</td>
</tr>
<tr>
<td>Low</td>
<td>32(62.7%)</td>
<td>172 (95.6%)</td>
<td>204 (88.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>51(22.1%)</td>
<td>180(77.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

High vs Low ($X^2$, OR, P) 41.4, 12.76, 0.00*

$X^2$ (chi-square) OR (odds ratio) P (p-value at 95% confidence interval) * (p-value significant)

4.5 The Frequency of A and non-A Blood Groups in the two Malaria Categories

The proportion of blood group A (56.9%) was higher in the severe (complicated) malaria than in uncomplicated category of malaria. In uncomplicated malaria category, blood group O was dominant (37.8%) followed by blood group A (35%).
As compared with uncomplicated malaria, blood group A has more than two fold (OR=2.45, 95% CI 1.30-4.61, P <0.001) increased chance of developing severe malaria than non-A blood group phenotypes. Therefore, blood type A has significantly (P < 0.001) increased risk of developing severe (complicated) disease than non-A blood types (Table 9).

Table 9. Frequency distribution of blood group A and non-A among two malaria categories

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29(56.9%)</td>
<td>63(35%)</td>
<td>92(39.8%)</td>
</tr>
<tr>
<td>Non-A</td>
<td>22(43.1%)</td>
<td>117(65%)</td>
<td>139(60.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>51(22.1%)</td>
<td>180(77.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

A vs non-A (X², OR, P) 7.92, 2.45, 0.005*

X² (chi-square) OR (odds ratio) P (p-value at 95% confidence interval) * (p-value significant)

When compared with each blood group, the risk of developing complicated malaria was significantly higher in individuals with blood group A than all other blood types. As compared with uncomplicated malaria the chance of getting complicated malaria in individuals with blood group A was nearly three (X²=7.34, OR=2.84, 95% CI=1.31-6.17, P=0.008) times higher than individuals with blood group O (Table 10).
Table 10. Odds ratio between blood group A and other blood groups for clinical state of malaria

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Complicated vs Uncomplicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X^2$</td>
</tr>
<tr>
<td>A vs B</td>
<td>5.59</td>
</tr>
<tr>
<td>A vs AB</td>
<td>3.98</td>
</tr>
<tr>
<td>A vs O</td>
<td>7.34</td>
</tr>
</tbody>
</table>

$X^2$ (chi-square value) OR (odds ratio) P (p-value at 95% confidence interval) * (p-value significant)

4.6 The Frequency of B and non-B Blood Groups in the two Malaria Categories

The proportion of blood group B was nearly equal among uncomplicated and complicated malaria categories. It was about 22.2% of the uncomplicated malaria and 21.6% of the complicated malaria and the observed difference was not statistically significant (P >0.05) (Table 11).

Table 11. Frequency distribution of blood group B and non-B among two malaria categories

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>11(21.6)</td>
<td>40(22.2%)</td>
<td>51(22.1%)</td>
</tr>
<tr>
<td>Non-B</td>
<td>40 (78.4%)</td>
<td>140(77.8%)</td>
<td>180(77.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>51(22.1%)</td>
<td>180(77.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

B vs Non-B ($X^2$, OR, P) 0.01 , 1.04, 1.00

$X^2$ (chi-square value) OR (odds ratio) P (p-value at 95% confidence interval)
4.7 The Frequency of O and non-O Blood Groups in the two Malaria Categories

Blood group O was the dominant type in uncomplicated malaria group. It was about 21.56% in complicated malaria and being blood group O significantly decreases the chance of having severe (complicated) malaria than non-O blood groups. Therefore, blood group O (OR=0.453, 95% CI 0.218-0.942, P=0.034) has less chance of developing a severe malaria infection than other non-O blood group phenotypes (Table 12).

Table 12. Frequency distribution of blood group O and non-O among two malaria categories

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>11(21.6%)</td>
<td>68(37.8%)</td>
<td>79(34.2%)</td>
</tr>
<tr>
<td>Non-O</td>
<td>40(78.4%)</td>
<td>112(62.3%)</td>
<td>152(65.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>51(22.1%)</td>
<td>180(77.9%)</td>
<td>231(100%)</td>
</tr>
</tbody>
</table>

O vs non-O (X², OR, P) 4.64, 0.453, 0.034*

<table>
<thead>
<tr>
<th>X² (chi-square)</th>
<th>OR (odds ratio)</th>
<th>P (p-value at 95% confidence interval)</th>
<th>* (p-value significant)</th>
</tr>
</thead>
</table>

When compared with other blood groups, blood group O has significantly less chance of developing severe (complicated) malaria; blood group A has nearly threefold and blood group B has nearly two fold increased risk of getting complicated malaria than blood group O (Table 13).
Table 13. Odds ratio between blood group O and other blood groups for clinical state of malaria

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Complicated vs Uncomplicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X^2$</td>
</tr>
<tr>
<td>O vs A</td>
<td>7.34</td>
</tr>
<tr>
<td>O vs B</td>
<td>3.82</td>
</tr>
<tr>
<td>O vs AB</td>
<td>1.43</td>
</tr>
</tbody>
</table>

$X^2$ (chi-square value) OR (odds ratio) P (p-value at 95% confidence interval) *(p-value significant)

4.8 Association between ABO Blood Groups and Severity of the Clinical state of Malaria

Of the total 231 malaria-infected patients, about 22.1% developed severe (complicated) malaria. The proportion of severe (complicated) malaria was higher in individuals with blood group A (31.5%) than other blood group phenotypes. The other blood group phenotypes proportions were B (21.6%), O (13.9%) and zero for blood group AB in complicated malaria category (Fig 5).

Among patients with severe (complicated) malaria, more than half (56.9%) was blood group A, and the remaining were 21.6% blood group B and 21.6% blood group O. The observed difference among the ABO blood groups with clinical state of malaria was statistically significant (P=0.016) that is there was a significant association between clinical state of malaria and ABO blood groups (Table 14).
As compared to uncomplicated malaria, the chance of having severe (complicated) malaria infection in patients with blood group A was nearly three (OR=2.84, 95% CI=1.31-6.17, P=0.008) times higher than individuals with blood group O. Individuals with blood group B have also higher chance of developing severe (complicated) malaria infection than individuals with blood group O phenotype (OR=1.72, 95% CI=0.68-4.28, P=0.026). Therefore, blood group A and B have more risk of developing severe (complicated) malaria than blood group O.
Table 14. Frequency distribution of ABO blood groups among two categories of malaria

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Total examined</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>92 (39.8%)</td>
<td>29 (56.9%)</td>
<td>63 (35%)</td>
</tr>
<tr>
<td>B</td>
<td>51 (22.1%)</td>
<td>11 (21.6%)</td>
<td>40 (22.2%)</td>
</tr>
<tr>
<td>AB</td>
<td>9 (3.9%)</td>
<td>0</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>O</td>
<td>79 (34.2%)</td>
<td>11 (21.6%)</td>
<td>68 (37.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>231 (100%)</td>
<td>51 (22.1%)</td>
<td>180 (77.9%)</td>
</tr>
</tbody>
</table>

$X^2, P$ 10.38, 0.016$^*$

$X^2$ (chi-square)  P (p-value at 95% confidence interval) * (p-value significant)
Chapter Five: Discussion and Conclusion

5.1 Discussion

Since the discovery of the ABO blood groups, numerous studies revealed the association between ABO blood groups and various diseases (Christine M. Cserti & Walter H. Dzik, 2007). Various investigators were looking for a relation between the ABO blood groups and malaria infection as well as the clinical state. However, those studies have failed to establish an exact link between the ABO blood groups and malaria infection as well as the severity of the disease.

In the present study, *P. falciparum* was the dominant parasite species with the relative proportion of 60.6% of the total 231 malaria cases. This is consistent with the report of FDRE/MoH that *P. falciparum* and *P. vivax* are the two dominant parasite species with the relative frequency of 60% and 40%, respectively in Ethiopia (FDRE/MoH, 2004).

The present study indicated that blood group A phenotype was dominant type among the study participants with the relative proportion of 38.9%. This finding contradicts with the fact that blood group O is dominant in the tropical region and other previous studies which reported blood group O was dominant in their study participants (Tekeste & Petros, 2010; Zerihun et al., 2011; Tadesse & Tadesse, 2013). This variation may be because of increased risk of blood group A patients to develop complicated malaria and/or due to issues related to sampling technique and small sample size of the present study.
Nearly half (46.4%) of *P. falciparum* infected individuals were blood group A and about (43.9%) of *P. vivax* infected individuals were blood group O. The observed difference between plasmodium species (*P. falciparum* and *P. vivax*) and ABO blood groups was not statistically significant that is malaria infection with *P. falciparum* or *P. vivax* species was not associated with specific ABO blood groups.

This finding contradicts with some studies reporting that malaria infection showed significant association with ABO blood groups with the highest proportion among individuals with AB blood group (Zerihun et al., 2011, Simon-Oke et al., 2016) and blood group A (Simiyu, 2013).

However, some other studies reported findings which are in agreement with the present study, that there was no significant association between ABO blood groups and prevalence of malaria (Montoya et al., 1994; Uneke, 2007). Also in agreement with the present study, absence of differences in the frequency of ABO blood groups among malaria infected individuals was suggesting that the effect of ABO blood groups on malaria infection was not statistically significant (Rowe et al., 2007).

The present finding showed that most (86.3%) of the severe (complicated) malaria cases were caused by *P. falciparum*. This agrees with other investigators reporting that *P. falciparum* was responsible for the most severe form of the disease and accounts for over 96% of all malarial infections in sub-Saharan Africa (Minja, 2013).

The present study revealed that about 95.6% of patients with uncomplicated malaria had low parasite density and about 37.3% of the complicated cases had high parasite density. This is in agreement with other findings that low parasitemia was observed among individuals with uncomplicated malaria (Tekeste & Petros, 2010; Zerihun et al., 2011).
As compared with uncomplicated malaria, we found that the chance of getting complicated malaria was higher among individuals with high parasite density than those with low parasite density. The probability of complicated malaria was nearly thirteen times higher in individuals with high parasite density than those with low parasite density.

Similarly, other studies found that individuals with severe malaria had significantly higher parasite count than those with uncomplicated malaria (Zerihun et al., 2011; Tadesse & Tadesse, 2013).

In the present study, blood group A and B showed more susceptibility to severe (complicated) malaria than blood group O. Higher proportion of individuals with blood group A (31.5%) and B (21.6%) were found to have severe (complicated) malaria than blood group O (13.9%). This is in agreement with previous studies which reported that individuals with non-O blood groups were more susceptible to develop severe *P. falciparum* infection than those with blood group O (Tekeste & Petros, 2010; Zerihun et al., 2011; Gayathri et al., 2013).

As compared to uncomplicated malaria, the chance of getting severe (complicated) malaria infection in patients with non-O blood group was higher than patients with blood group O. Blood group A was about three times more prone to develop severe (complicated) malaria and blood group B has a two-fold increased risk of developing severe infection than blood group O. This finding also agrees with previous investigators who reported that non-O blood groups have greater risk of developing severe disease (Fry et al., 2008).

Similarly, patients with blood group ‘B’ have a four-fold increased risk (Panda et al., 2011) and patients with blood group A have almost three times more risk (Pathirana et al., 2005) of developing severe malaria than blood group O.
In this study, individuals with blood group O had significantly less risk of developing severe (complicated) malaria infection than individuals with other blood groups. This is in agreement with a study conducted among Malian children, that blood group "O" confers significant protection against severe malaria compared with the non-"O" blood groups (Rowe et al., 2007).

It also agrees with other researchers report, that the protective effect of blood group O was demonstrated with a decreased risk of developing severe malaria than other blood groups (Panda et al., 2011; Amodu et al., 2012).

The mechanism by which blood group O confers protection against severe malaria compared to other blood groups is not fully understood. However, trisaccharides of ‘A’ and ‘B’ blood group are presumed to act as receptors and function as an important factor for rosetting (Barragan et al., 2000) and erythrocyte rosetting (binding of one infected RBC to two or more uninfected RBCs) is linked to the pathogenesis of severe malaria (Fry et al., 2008).

Rosetting has been established as a *P. falciparum* virulent factor, the expression of which is modified by a variety of host factors which include the ABO and Rh blood type (Christine M. Cserti & Walter H. Dzik, 2007). However, blood group antigens A and B are not expressed in blood group O individuals and because of it, rosettes formed by blood group O are suggested to be smaller and easily disrupted than rosettes formed by blood group A, B or AB erythrocytes (Singh, Urhekar, & Singh, 2015).

In addition, parasitized erythrocytes form rosettes more readily if they are belonging to blood group A or B than blood group O isolates (Senga et al., 2007) and rosettes formed in blood types A and B are larger, tighter and stronger than those formed in individuals with blood type O (Qijun et al., 2000).
Therefore, the protective effect of blood group O against severe (complicated) malaria may be because of the absence of both trisaccharides A and B on the surface of RBCs belonging to blood group O by which the risk of developing severe disease may be decreased.

None of the individuals with blood group AB developed severe (complicated) malaria in this study. This finding contradicts with other studies reported blood group AB was more susceptible to severe (complicated) malaria than other blood group (Pathirana et al., 2005; Fry et al., 2008; Tekeste & Petros; 2010; Zerihun et al., 2011).

However, the present study finding may not indicate that blood group AB absolutely provides protection against severe (complicated) malaria because; the proportion of blood group AB among the study participants was very small; only 9 individuals were having AB blood group phenotype. Therefore, it is not safe to conclude that blood group AB has protective effect against severe malaria infection.

The present study demonstrated that more than half (56.9%) of individuals with complicated malaria were blood group A and blood group A was significantly associated with complicated malaria as compared with non-A phenotypes. This is in agreement with other's report that there was high percentage of blood group A and low percentage of group O individuals in the severe malaria category than in uncomplicated malaria. (Tekeste & Petros, 2010)

According to the present study, being blood type A significantly increases the risk of developing severe disease than non-A phenotypes. As compared with uncomplicated malaria, blood group A has more than two fold increased chance of developing severe malaria than non-A blood group phenotypes.
This is in agreement with other investigators who reported that blood type A is most often affected by severe (complicated) malaria (Qijun et al., 2000). Similarly, research done in northwestern Ethiopia indicated that severe (complicated) malaria occurs more frequently in individuals with blood group A than other blood groups (Tadesse & Tadesse, 2013).

This finding that blood group A is more susceptible to severe (complicated) malaria than other blood groups may be because of other coexisting factors in addition to ABO antigens that play important role in the pathogenesis of severe (complicated) malaria, complexity of the interaction between the parasites and host immune responses and impact of other RBCs polymorphisms.
5.2 Conclusion

This study revealed that ABO blood groups have no association with malaria infection caused by either *P.falciparum* or *P. vivax* species. However, significant association between ABO blood groups and severity of the clinical state of malaria was observed. Individuals with blood group A had increased risk of developing severe (complicated) malaria than individuals with other blood groups, whereas individuals with blood group O had significantly less risk of developing severe (complicated) malaria than individuals with other blood group phenotypes.
5.3 Recommendation

- Further in-depth molecular level (cytology or culture) studies are recommended to clearly establish the biological role of the ABO blood group antigens during *P. falciparum* and *P. vivax* infection and pathogenesis of malaria.
- In addition to ABO antigens, other co-existing RBCs polymorphisms should be deeply investigated to differentiate exact role of ABO antigens during malaria infection and pathogenesis.
- Based on the findings of present study, special attention is required to be given to individuals with non-O blood groups during clinical management and control of malaria.
REFERENCES


President’s Malaria Initiative Ethiopia Malaria Operational Plan FY 2016. (2016), 1–76.


Vang Petersen, J. E. (2016). The Pathogenic Role of Plasmodium falciparum Erythrocyte Membrane Protein 1 Interacting With Endothelial Protein C Receptor.


### Part-I Socio-demographic characteristics

1. **Gender**
   - □ Male
   - □ Female

2. **Age**—— years

3. **Religion**
   - □ Orthodox
   - □ Protestant
   - □ Muslim
   - □ Other specify——

4. **Ethnicity**
   - □ Gamo
   - □ Gofa
   - □ Amhara
   - □ Konso
   - □ Wolaita
   - □ Other specify——

5. **Marital status**
   - □ Single
   - □ Married
   - □ Divorced
   - □ Widowed

6. **Educational status**
   - □ Illiterate
   - □ Reading and writing
   - □ Primary school
   - □ Secondary school
   - □ College/Diploma
   - □ University/Degree
   - □ Postgraduate

7. **Occupational status**
   - □ Employed full-time
   - □ Employed part-time
   - □ Retired
   - □ Unemployed
   - □ Casual worker
   - □ House wife

8. **Average monthly income**——(birr)
Part-II Clinical and laboratory findings

Clinical findings (filled by nurses after physician diagnosed)

1. Blood pressure (mmHg) -----------------
2. Impaired consciousness A. Yes B. No
3. If yes for Q2, Glasgow coma scale score -----------------
4. Signs of respiratory distress (acidotic breathing) A. Yes B. No
5. Abnormal bleeding A. Yes B. No
6. Yellowish discoloration of sclera A. Yes B. No
7. Clinical diagnosis (from patient card or individual folder) -----------------

Results of laboratory investigation (filled by medical laboratory technologists)

1. Identified plasmodium species
   - ☐ P. falciparum
   - ☐ P. vivax
   - ☐ Both P. falciparum and P. vivax
   - ☐ Others
2. Parasite density
   - ☐ +
   - ☐ ++
   - ☐ +++
   - ☐ ++++
3. Hemoglobin concentration (gm/dl) -----------------
4. Random blood sugar (mg/dl) -----------------
5. Blood group -----------------
ANNEX 2

QUESTIONNAIRE (Amharic Version)

የግል የግል የግል የግል የግል የግል የግል የግል የግል

1. የታዩን የታዩን የታዩን የታ几何

2. እድመ ዕድመ ዕድመ ዕድመ

3. ከወጣት ከወጣት ከወጣት ከሚያጥስ ከሚያጥስ ከሚያጥስ ከሚያጥስ

4. ዓይጢ

5. ይህን ቤት ይህን ቤት ይህን ቤት

6. ከትምህርት የትምህርት የትምህርት የትምህርት

7. ይህን ቤት ይህን ቤት ይህን ቤት

8. ከአማካይ የአማካይ የአማካይ የአማካይ
ANNEX 3

Information and consent/assent form (English version)

Information sheet

Addis Ababa University, College of Health Sciences, School of Medicine, Department of Medical Physiology

Investigator: Gizachew Girma (BSc.)

Supervisor: Dr. Diresibachew Haile (Ph.D)

Study title: Association of ABO blood groups with *P. falciparum* and *P. vivax* malaria infection and severity of the clinical state at Arba Minch General Hospital, Gamo Gofa, Southern Ethiopia.

Dear participant! Here, I the undersigned, at Addis Ababa University College of Health Science, Department of Medical Physiology. Graduate Study Program, currently I will be undertaking research on a topic mentioned above. For this study, you will be selected as a participant and before getting your consent, you need to know all necessary information related to the study which will be detailed as follows.

Introduction: Privacy is the state of being free from intrusion, and in the context of health care it concerns the responsibility of a care provider to protect a client’s from any disclosure (i.e., discovery by others), even unintentional of personal health data, by providing security to the patient and the patient’s records.
Confidentiality, in contrast, is the limiting of information to only those for whom it is appropriate. Therefore this information sheet briefly provides the necessary guide to be considered during the study.

**Study purpose:** the main aim of this study is to assess the association between ABO blood group and *P.falciParum* and *P.vivax* malaria infection and severity at Arba Minch General Hospital.

**Procedure:** If you agree to participate in the study, you will first consult with a medical or clinical officer and he/she will refer you to the laboratory for your blood sample to be taken.

You will be seated and blood will be drawn by putting a needle into a vein in your arm. One small tube of blood about 2ml will be taken. This will take about five minutes.

**Risks and discomfort:** Participation in this study will not cause more discomfort and no need for extra sample other than a sample taken for the diagnostic purpose. But, there could be minor pain and a bruise around the site on your arm where the needle is inserted to collect the blood sample. This is rare when blood is drawn by trained personnel. If such a bruise develops, it will resolve without treatment within a few days and is rarely painful.

There is also a risk of excessive bleeding if you suffer from a blood clotting disorder or are taking medication that inhibits blood clotting. If either of these conditions applies to you, it would be best if you do not donate blood for this study, if not, there is no major risk in participating in this research.

**Benefits:** There is no benefit in participating in this study. However, you will have the chance to know your blood group and hemoglobin concentration from the laboratory result.
And if your result reveals any incidental health problems that need immediate treatment, you will be referred to an appropriate health facility.

**Incentive:** There is no financial or material incentive in participating in this study.

**Confidentiality:** The information that will be collected from this research project will be kept confidential. Information about you will be stored in a file, which will not have your name on it, but a code number will be assigned to it and will not be revealed to anyone except the principal investigator.

**Participant Rights:** Your participation is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. Also, you have the right not to answer any questions you do not want to. You may also withdraw from the study at any time and there will be no consequences if you refuse to participate in the study.

**Person to contact:** If you need more explanation about the study and need to ask any question, you can ask at any time, and you can contact:

Principal investigator: Gizachew Girma,

Cell phone: +251-928972080

E mail:gizachewgirma96@gmail.com
Consent form

Name of the study participant ………………………

Code number……………………

I have clearly been informed about the research purpose that it aims to assess an association between ABO blood groups and malaria infection and severity of the clinical state. I have understood that participation in this study is entirely voluntarily. I have been told that my answers to the questions will not be given to anyone else and no reports of this study ever identify me in any way. I have also been informed that my participation or non-participation or my refusal to answer questions will have no effect on me and participation in this study does not involve major risk. If I have any questions about the study or about my rights as a study participant, I will contact the principal investigator Gizachew Girma.

Respondent’s signature………………

Interviewer name……………………signature………………date………………

Thank you for your cooperation