COLLEGE OF MEDICINE AND HEALTH SCINCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

PREVALENCE OF MALARIA, ITS ASSOCIATION WITH ABO BLOOD GROUP AND IT'SASSOCIATED RISK FACTORS AMONG ACUTE FEBRILE PATIENT ATTENDING WORABE HEALTH CENTER, SILTE ZONE, SOUTH ETHIOPIA

BY: 1. ABEBE TALEMA

2. BEKELE MERSHA

3. HAYAT MUSTEFA

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WOLKITE UNIVERSITY, COLLEGE OF MEDICAL AND HEALTH SCIENCES, SCHOOL OF MEDICAL LABORATORY SCIENCES

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BY: 1. ABEBE TALEMA

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ADVISORS: Mr. LEMA WORKINEH (MSC,Ass.prof)& Mr. DEREJE ABEBE (Bsc,Msc)

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ABBREVIATIONS AND ACRONYMS

ABO Blood Group A, B and O

BF Blood Film

BG Blood Group

RBC- Red Blood Cell

ITN Insecticide Treated Net

IRBC Infected Red Blood Cell

QBC Quantitative Buffy Coat

SNNP- South Nation Nationalities of People

SOP- Standard Operating Procedure

SPSS Statistical Package for Social Science

WBC- White Blood Cell

WHO- World Health Organization

WKU- Wolkite University

ABSTRACT

Background: *Malaria is caused by an obligate, intra cellular protozoa parasite of genus plasmodium species. The virulence of plasmodium species has been associated with the capacity of the infected RBC to adhere to uninfected RBCs, leading to rosetting of cells.The majority of study was focused only on prevalence and risk factors.But, no more study conducted for further investigation about the significance of ABO blood group and parasite density on prevalence of malaria infection.Therefore, this study was aimed at filling the above gap in the study area.*

Objective*: The objectives of the study was to determine the prevalence of malaria infection and its association with ABO blood group among acute febrile patients attending at Werabe health center from March 2023 to August 2023 in silte zone, werabe town, Ethiopia.*

Methodology: *A cross sectional study design was conducted to determine the association of malaria infection with ABO blood group antigens and it's associated risk factors at Worabe health center from May to July 2023.384 participants was included in the study by using convenient sampling Technique . Data was entered and analysed by SPSS and finally analysed by bivariate and multivariate logistic regression . Finally, factors with P values ≤.05 were considered statistically significant.*

Result ;*Out of a total of 384 patients who visited at werabe health center for medical attention, 56(14.6%)are found to be infected with plasmodium parasites as detected by microscopy, among which 42(12.3%), 11(7.6%), and 3(0.5%) are belong to Plasmodium vivax, Plasmodium falciparum and mixed infection respectively. All patients examined for malaria are also tested for ABO blood groups. Accordingly,48.6%,27.1%,21.5%,and 2.6% are found to be blood types of O,A,B and AB, respectively(x^2=4.66,P=0.86) .Out of 56 infected patients,42.8% 28.6%,19.6% and 8.9% are plasmodium infected patients with blood groups A, O, B and AB respectively .*

Conclusion:*The overall prevalence of malaria was 14.6% and Plasmodium vivax is the dominant species in the study site . This study revealed that individuals of blood group A are more susceptible to plasmodium infection.*

Key words;*Acute febrile patients,malaria , ABO blood groups association, worabe healrh center,Ethiopia*

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Malaria is a major public health problem in worldwide and causes high morbidity and mortality. It is a vector borne infectious disease caused by a eukaryotic Protista of the genus Plasmodium. The most medically important species are Plasmodium ovale*, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium Knowlesi* and the disease is transmitted by *female Anopheles mosquitoes* which carry infective sporozoite stage of Plasmodium Parasite in their salivary glands[1].The major or the primary malaria vector incriminated in Ethiopia is *Anopheles arabiensis*; and others are *A. pharoensis, A. funestus and A. nili*.An *Anopheles arabiensis* is the only species from the *An.gambaie* complex known to be prevalent across malaria endemic areas in Ethiopia[2]

Households in close proximity to breeding sites have higher mosquito densities and increase the risk of transmission and the rapid rise in development work and stagnant water at construction sites in urban area have also leaded to the rise in the incidence of malaria[3].

Malarial infection begins when a person is bitten by an infected female anopheles mosquito and Plasmodium spp (species) parasites in the form of sporozoites are injected into the bloodstream. The sporozoites travel to the liver, multiplying asexually over the next 7–10 days. The vesicles eventually disintegrate, releasing the merozoites to enter the bloodstream where they invade and multiply in erythrocytes.When the cells burst, the parasites invade more erythrocytes. In some infected blood cells, instead of replicating asexually, the merozoites develop into sexual forms (gametocytes), which circulate in the bloodstream and are ingested during mosquito bites[4].

Pathogenetic Characteristics of Severe Malaria are Cytoadherence, the ability of parasites to adhere to the vascular endothelium, Mature forms of parasites (asexual stage and gametocytes) can adhere to the vascular endothelium of several organs (lung, heart, brain, lung, liver, and

kidney) causes different clinical complications which include about malaria paraxosym like cold stage ,febrile stage and sweate stage and other cerebral malaria, severe anemia, jaundice, renal failure, metabolic acidosis, hypoglycemia, and acute respiratory distress syndrome and another pathogenesis is Rosetting ,Rosetting is one of the forms of cytoadherence of late stages IRBC to non-parasitized red blood cells and /or platelets[5].

In the laboratory, malaria is diagnosed using different techniques. conventional microscopic diagnosis is the gold standard technique performed by staining thin and thick peripheral blood smears.There are also other techniques, like quantitative buffy coat (QBC) , rapid diagnostic antigen tests molecular techniques and antibody tests. .Microscopic diagnosis remains the standard of diagnosis in health centers and hospitals of different levels[5].

There are current interventions for malaria prevention, which include indoor residual spraying,insecticide treated nets(ITNs), intermittent preventive therapy , presumptive remedy, and education. primaquine is the WHO-recommended treatment for relapse prevention where *plasmodium vivax* predominate and artemisinin –based combination therapies (ACTs) are the recommended treatments for uncomplicated *plasmodiumFalciparum*. [6].

The ABO blood group was the first human blood group discovered in 1901 by Landsteiner followed by Rh blood group in 1941 ,ABO blood groups are the most medically important blood types that are very useful in assuring safe human blood transfusions[7].

The ABO blood groups are four principal types: A, B, AB, and O which have two antigens and two antibodies. The specific combination of these four components determines an individual's blood group type. The invasion of RBCs is mediated by parasite adhesins and binding receptors on the red cell surface alongside a repertoire of signaling pathways within the erythrocyte, which alters red cell biophysical properties to and facilitate invasion . Studies on RBC factors during the process of parasite invasion show that specifc receptor-ligand interactions facilitate RBC binding involved in blood group antigens .Parasite invasion rate into erythrocytes is dependent on erythrocyte blood group antigens[8].

Blood group antigens A and B are trisaccharide's attached to variety of glycoprotein and glycolipids on the surface of erythrocytes, and these trisaccharide's are thought to act as receptors for rosetting legands. However, blood group antigens A and B are not expressed in group "O" individuals. As a result, 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[9].

A number of glycoproteins have been reported to play a role in sequestration of malaria-infected erythrocytes such as a leukocyte differentiation antigen which is widely expressed on endothelium, platelets and leucocytes. Studies have reported that P. falciparum rosettes that form in group O cells are smaller and more easily disrupted than those formed in groups A, B and AB erythrocytes. Reported that blood group O protects against severe P. falciparum malaria through reduced rosetting mechanism.[10]

Generally Malaria is a major public health problem in worldwide and causes high morbidity and mortality.It is a vector borne infectious disease and transmitted by *female Anopheles mosquitoes* which carry infective sporozoite stage of Plasmodium Parasite in their salivary glands.Households in close proximity to breeding sites have higher mosquito densities and increase the risk of transmission and the rapid rise in development work and stagnant water at construction sites in urban area have also leaded to the rise in the incidence of malaria and A number of glycoproteins have been reported to play a role in sequestration of malaria-infected erythrocytes such as a leukocyte differentiation antigen which is widely expressed on endothelium, platelets and leucocytes. A blood group O protects against severe P. falciparum malaria through reduced rosetting mechanism.

1.2 Statement of the problem

About 3.3 billion people – half the world's population – are at risk of malaria due to the main causative parasite, Plasmodium falciparum. Over 200 million cases of malaria occur each year, 90% of which occur in Africa, and 655 000 people died from the disease in 2010, making it one of the world's most important health problems[4].

About 694 million people in Africa are estimated to be at risk of malaria, which represents 21% of the global population at risk. According to the September 2015 WHO weekly epidemiological record, there were about 214[11]. About 90% of all malaria deaths in the world today occur in Africa south of the Sahara. In all malaria-endemic countries in Africa, 25–40% (average 30%) of all outpatient clinic visits are for malaria (with most diagnosis made clinically). In these same countries, between 20% and 50% of all hospital admissions are a consequence of malaria.In Africa, most cases of malaria are diagnosed on the basis of clinical symptoms and treatment is presumptive, rather than based on laboratory confirmation. Moreover, malaria parasitaemia is common among clinic attendees in many endemic areas, so that a positive laboratory result does not necessarily mean that the patient is ill with malaria[12].

In Ethiopia, approximately 68% of the land mass of the country have favourable conditions for malaria transmission, and 60% of the population are at risk of malaria . The transmission of malaria is unstable and seasonal, usually characterized by frequent focal, and sometimes large scale epidemics . Plasmodium falciparum (70%) and Plasmodium vivax (30%) are the common causes of malaria[13].In Ethiopia, malaria is one of the 10 top leading causes of morbidity and mortality among children. It is common in children, which ranges from 16% to 54% in the country. It remains a public health problem in the country due to low coverage of insecticide-treated bed nets (ITN), low coverage of indoor residual spraying, drug resistance to malaria, insecticide resistance of malaria vectors, poor access to health care, migration of people from malaria endemic areas to non-endemic areas, and false microscopic results[1].

In view of a heavy Burden placed on human health due to malaria many investigation have been conducted to find out whether or not ABO blood group antigens are associated with susceptibility resistance, or severity of *plasmodium falciparum* malaria. Some studies reported the absence of significant association between plasmodium and ABO antigens. On the other hand

other studies have shown that high frequency of malaria episodes have been observed among blood group A individuals as compared with other blood groups individuals'[14]. Blood group A was significantly associated with severe malaria compared with blood group O as well as with a trend towards higher parasitaemia. Blood group O was seen more often in the mild group and those with malaria and blood group A had lower haemoglobin levels and a higher risk of coma than infected patients with other blood group types[15].

There was no more study conducted for further investigation about the significance of ABO blood group on prevalence of malaria infection furthermore, so no enough data on malaria prevalence, risk factors, or parasite density among participant in worabe health center. Thus, the aim of this study was determine the prevalence of malaria,its association with ABO blood group and associated risk factors among acute febrile patient in Worabe health center.

1.3 Significance of the study

Knowing the prevalence of malaria infection and association with ABO blood group and other related risk factors among in the community is very important for zonal and werada health office of werabe town in order to intervene accordingly. Indicating which type of Plasmodium species are the most prevalent in the study area is essential for intervention strategies. The results of the study may also help the local Health Centers and concerned health offices to know the burden of malaria and prevalent species in the study area and to plan well-organized malaria prevention and control program. It also provide information on the prevalence of malaria parasite infection and its association with ABO blood group in the community of study area and furthermore, the study will be used as recent information for those who need to conduct further investigation in the area.

CHAPTER TWO

2. LITRETURE REVIEW

To determine whether ABO blood group antigens are connected to malaria susceptibility, resistance, or severity, numerous studies have been carried out. The most severe forms of human malaria and virulence associated with parasite reproduction rate and erythrocyte invasion mechanism are linked to Plasmodium infections. The relationship between ABO blood types and malaria susceptibility has been studied by several researchers; however, the results have been contradictory[14].

The large numbers of severe malaria cases are also reported among blood group 'A' individuals. Malaria parasitaemia was higher among the males (83.3%) than females (75.0%). Blood group 'A' individuals may be more susceptible to malaria attack due to high chance of rosette formation especially for *P. falciparum* infection[14].

According to retrospective study conducted by Zhangx etl in 2015 at Zhejiang university, China, Overall, 90 (90.91%) of the patients were positive for Plasmodium falciparum, 8 (8.08%) were infected with Plasmodium vivax, and only 1 (1.01%) was infected with Plasmodium malariae. The most common blood group among the participants was group O (38.38%) followed by blood groups A, B, and AB, with 32.32%, 22.22%, and 7.07% cases, respectively. There was no significant relationship between the prevalence of malaria and ABO blood types according to the study[16].

According to retrospective case control study conducted by Herrera NM etl in 2009 at zone of Apartado, Colombia a total sample of 92 patients was obtained, 53% with severe malaria and 46% with uncomplicated malaria. Of the patients with diagnosis of severe malaria 59.2% were women. There more frequent parasite species was P. falciparum. Severe malaria was more frequent among patients classified with blood group O (65.3%)[9].

A study associating malaria parasitemia with ABO blood groups among residents of Warri, Nigeria conducted by otajevwo DF in 2013 reported that 6.9%, 19.0%, 20.7% and 53.3% of total of 174 whole blood samples processed belonged to blood groups AB, B, A and O

respectively, and 138 (79.3%) of total sample size were infected with malaria parasites of which P. falciparum was the predominant species[17].

According to cross-section study conducted by Tadesse H and Tadesse K in 2013 at Felegeselam health center, Northwestern Ethiopia a total of 398 acute febrile patients, 201(50.5%) were found to be infected with plasmodium parasites. Of which 194(48.74%) and 7(1.76%) belong to P. falciparum and P. vivax, respectively. The distribution of ABO blood groups was O (46%), A (27.1%), B (23.1%) and AB (3.8%). The percentage of severe malaria with respect to blood group A, B, AB and O was found to be 40,34.1,14.3 and5.1% respectively[18].

A study conducted by Area BD etl in 2011 at Dore Befano health center, Southern Ethiopia, showed that out of a total of 269 participants,178(66.2%) febrile patients were found to be infected with plasmodium parasites, among which 146(54.3%),28(10.4%), and 4(1.5%) belong to P. falciparum, P. vivax and mixed infections, respectively. All febrile patients were also tested for ABO blood groups and 51.3%,23.5%,21.9% and 3.3% were found to be blood types of O, A, B and AB, respectively. The proportion of A or B but not O phenotypes were higher in individuals with P. falciparum as compared with P. vivax[14].

According to study conductedTekezte Z and Petros B in 2010 at Awash, Metehara and Ziway, Ethiopia 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%). 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[19].

According to cross sectional study conducted by Tazebew B and Munshea A in 2021 atMekaneeyesus Primary Hospital, Estie District, northwest Ethiopia, the overall prevalence of malaria was 8.5%; Plasmodium vivax (5.6%) was the most predominant, followed by P. falciparum (2.3%), and mixed infection of the two species (0.5%). In this study, being male , under-five years of age , rural residence and failing to use bed were significantly associated with the risk of malaria. Most (14.6%) of malaria-positive cases were among individuals with

blood group “A,” while the least numbers of cases were among subjects with blood group “O.” Individuals with blood group “A” were about four times at risk of malaria as compared to individuals with blood group “O”[20].

Another study was conductedby Alemu A etl in 2011 at urban area malaria and associated risk factors in Jimma town in 2011. From the total of 804 participants in the current studies only 42 (5.2%) were positive for malaria parasites, *Plasmodium falciparum, Plasmodium vivax* and mixed infection accounted 26.2%, 71.4%, and 2.4% respectively. High malaria prevalence rate was observed under five children (11%). Those do not use insect side treated bed nets (ITN) were more likely to be infected with malaria compared with those who use ITN, living in the area where stagnant water exist and its distance of existence is less than 1km from the house were more likely to be infected with malaria parasites compared to those who live away from stagnant water at a distance greater than 1 km [21].

According to the recent six years report of Federal Minister of Health malaria burden in Amhara in 2004 by Fantahun M *et al*. Amhara regional state is the second highly infected next to Oromia, with malaria border compared to rest of largest region southern nations, nationalities and peoples region (SNNPR) and Tigray [22].Therefore, literatures regarding the association between ABO blood groups and malaria infection as well as severity of the disease were not clearly elucidated .

2.1 Conceptual framework

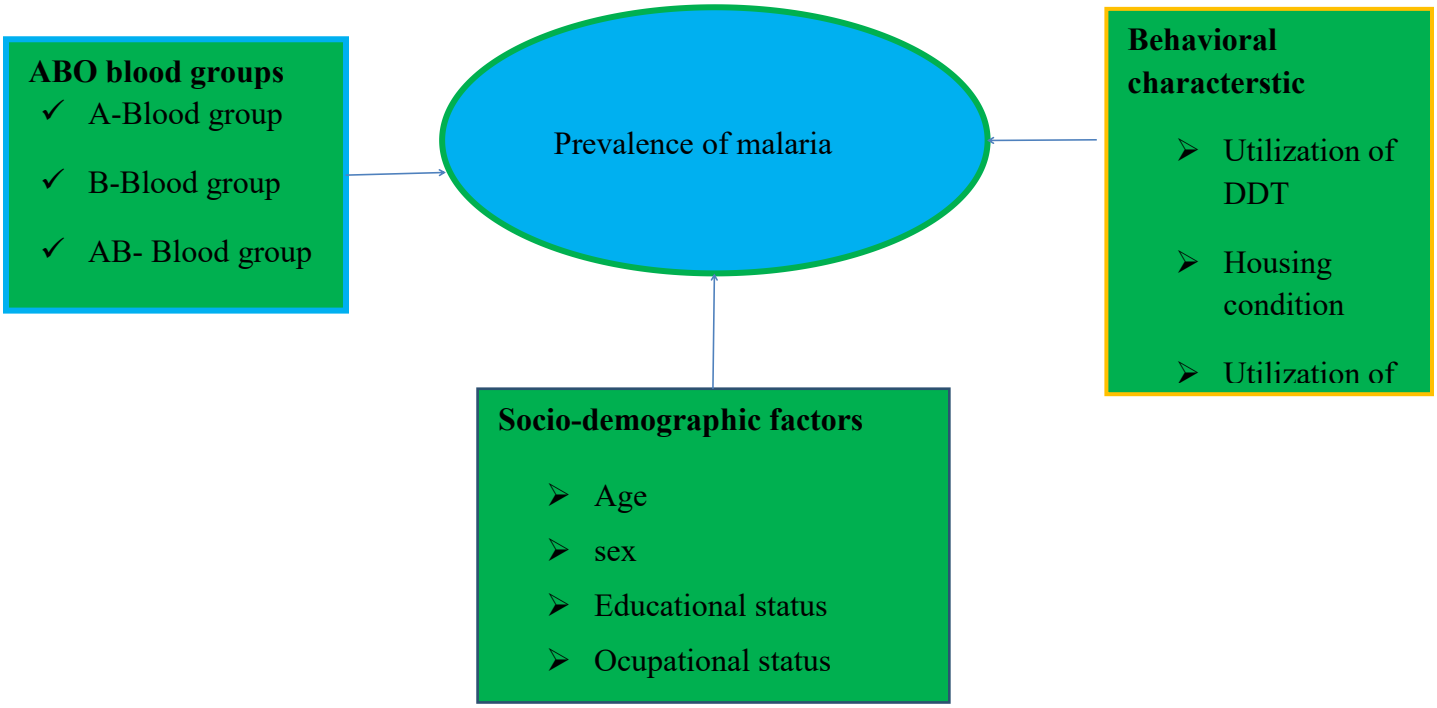

Figure 1 conceptual framework for prevalence of malaria infection and its association factors including of ABO blood group

CHAPTER THREE

3. OBJECTIVES

3.1 General objectives

- To assess prevalence of malaria, its association with ABO blood group and associated risk factors among acute febrile patients attending at Worabe health center, silte Zone, Southern Central Ethiopia. from May 03, 2023 to August 22 ,2023

3.2 Specific objectives

- To determine the prevalence of malaria parasite infection among patients attending Werabe health center during study period
- To determine the distribution of ABO Blood Group during study period
- To determine the association of ABO blood groups within malaria infection during study period
- To determine Associated risk factors of malaria infection during study period

CHAPTER FOUR

4. METHODS AND MATERIALS

4.1. Study Area

The study was conducted in Worabe health center ,worabe town,silte Zone. The study site is found in Silte zone, the town is located 158 km southwest of Addis Ababa, the capital city of Ethiopia. It has an average annual temperature of 25 °C and an average rainfall of 1244 mm. The attitude has an elevation between 1910 and 1935 meters above sea level. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia, Werabe town has a total population of 750,398 of whom 364,108 were males and 386,290 females.

4.2 Study design:

A cross-sectional study was conducted to assess the prevalence of malaria, its association with ABO blood group antigens and assaciated risk factors among patients attending Werabe Health center.

4.3. Study period: The study was conducted from May. 15 to August 20

4.4 Population

4.4.1 Source of population:

All acute febrile patients who were attend at Werabe health center.

4.4.2 Study Population:

Our study populations were patients who had requested for blood film examination for malaria diagnosis at werabe health center from May 5 to July 30

4.5 Inclusion and Exclusion criteria

4.5.1 Inclusion criteria

- ✓ All Patients who were suspected to have malaria and those who will volunteer to give blood specimen for malaria diagnosis and ABO blood group.

4.5.2 Exclusion criteria

- ✓ Individuals who cannot communicate due to impairment or sever sickness like coma, and mentally sick people were excluded from the study
- ✓ Those who currently on anti-malaria drugs within 42 days prior to data collection were excluded since it is considered that the parasite is eliminated from the blood within 42 days of treatment, and re-infection can occur after that time[23].

4.6 Sample size and sampling technique

4.6.1 Sample size determination

For determination of total sample size, we can use the formula: $n=z_{\alpha}^2pq/d^2$

Where,

n = total sample size

z=the standard normal variation at 95% confidence interval and, $\alpha=0.05$

d^2=margin of sampling error (d=0.05)

p=population proportion possessing the characteristic.

Due to lack of higher malaria prevalence in the previous research in our study area, the maximum prevalence (50%) was used to determine the sample size. Since higher prevalence rate is the best to do the association between malaria and ABO blood group

So, we take p=50%, w=0.5, with 95% confidence interval.

P=50% = 0.05

q=1-p, q=1- 0.5=0.5

$n= (1.96)^2\ 0.5(0.5)/\ (0.5)^2$, n=384

4.6.2 Sampling Technique

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

4.7 Variables

4.7.1 Dependent variables

- ✓ Prevalence of Malaria infection

4.7.2 Independent variables

- ✓ Age
- ✓ Sex
- ✓ Religion
- ✓ Ethnicity
- ✓ Occupation status
- ✓ Monthly income
- ✓ Housing condition
- ✓ Utilization of bed net
- ✓ ABO blood group

4.8 Data collection procedures

Socio-demographic characteristics and other required information were collected from the participants by face-to-face interview using English version of the structured questionnaire like age, presence of mosquito breeding site, occupational status, resident status, bed net utilization status, house structure and others .Capillary blood collection was performed by using aseptic techniques. Patient identification, specimen labeling and microscopic examination were done carefully. Laboratory investigation results was recorded by using well-structured report format.

4.9 Laboratory analysis

4.9.1 Sample collection and sample processing

Before collecting blood sample, explanation about the study was given and written informed consent was obtained from every study participants including the guardians of children. Capillary blood was collected by finger pricking using sterile disposable lancet. Heel punctures was used for infants. Immediately, thin films were spread on grease free, frosted end, labeled slide using a smooth-edged slide spreader. Thick films were also prepared on the same slide and

allowed to dry. Thin film was fixed with absolute methanol. The blood film was stained with 10% Giemsa for 10 minutes. Then the blood film was examined by laboratory technologist using an oil immersion microscope objective (100X) and the result was noted on the data collection format.At least 100 fields was examined before designating a sample 'negative', or more accurately, 'no malaria parasites seen'. .

To quantify malaria parasites against WBCs: on the thick smear, tally the parasites against WBCs, until 200 or 500 parasites were counted then the result expressed as parasites per microliter of blood, using the WBC count if known, or otherwise assuming 8,000 WBCs per microliter blood.

Parasites/microliter blood= (parasites/WBCs) × WBC count per microliter or 8,000.

The parasitemia was then divided into 4 categories: low (1000 parasites/µl of blood), moderate (1000-4999 parasites/µl of blood), high (5000-99999 parasites/µl of blood), and hyperparasitemia (100000 parasites/µl of blood [24].

4.9.2 ABO blood group phenotyping

ABO blood group was typed by agglutination using commercial antisera (direct method) two drops of whole blood placed into different places of grease free clean glass slide on which a few drops of antisera for blood group A and B will be applied. The blood and the antisera will be mixed with separate applicator stick. The slide will then be tilted to detect for agglutination and the result recorded accordingly.

4.11 Quality assurance and Quality control

4.11.2 Quality control

Internal procedural controls were included in the test. . Moreover 10% of positive and negative slides was taken and blindly checked by another laboratory professional.

Pre-analytical phase

- Good quality and well-defined reagent was used.
- The patient was instructed on how to collect specimen

Analytical phase

- SOP was used throughout the study period to avoid personal errors; professionally skillful laboratory personnel will examine randomly selected slides.

Post–analytical phase

- The result was checked before giving to the patient
- The result was recorded carefully and the data was analyzed appropriately**.**

Properly interpreting of hemagglutination during ABO phenotyping

4.12 Data entery and analysis

Data was entered and analyzed by SPSS software. Socio demographic parameters and the prevalence rate were calculated using descriptive statistics. Chi-square (X^2) is used to determine the association between ABO blood groups and malaria infection. Binary logistic regression was used to analyze the associated risk factors of malaria. After performing bivariate logistic regression analysis, variables with a P value of .25 were adjusted using a multivariate logistic regression model by stepwise variable selection to control for possible confounding factors. Finally, factors with P values ≤.05 were considered statistically significant.

4.13 Ethical Consideration

Ethical issues were considered in all steps of research process. A permission letter to do the research on the prevalence of malaria and its association with ABO blood group was obtained from Wolkite University Department of Medical Laboratory Science. The study subjects were informed about the aim and objectives and invited for voluntary participation in the study. Written informed consent was obtained from participants (age ≥18 years old) and oral assent was obtained from guardians /parents/ of the participant in case of aged less than 18 years. After permission was obtained, participants were interviewed and blood sample was collected. Confidentiality was kept by using codes instead of names for all participant-related data.The result was given to the respective health personnel for treatment.

4.14 Operational definition

Low parasitemia -when number of parasites is less than 1000 per microliter of blood

Moderate parasitemia-when number of parasites is between 1000 -5000per microliter of blood

High parasitemia- when number of parasite is between 5000-100,000per mirolitre of blood

Hperparasitemia- when the number of parasites is greater than 100,000per microliter of blood [24]

4.15 Dissemination plan of the study finding

The finding of the study will be presented and submitted to School of Medical Laboratory and College of Medicines and health science, wolkite university.

CHAPTER FIVE

5. RESULT

5.1 Socio-Demographic Characteristics of the Study participants

There were 182 (47.4%) males and 202 (52.%) females among the 384 patients who participated in the study. The participants in the study ranged i from 1 months to 73 years, with an average age of 17.7 years and a 4.46 year standard deviation. The majority of the participants came from urban areas 268(69.8%), while the rest 116(30.2%)came from rural areas. Regarding to ethnicity, majority of them were silte 374(97.4%). More than half of the respondents were followers of Muslim 307 (79.9%) and the rests were orthodox 68 (17.7%) and protestant 9 (2.3%). Regarding occupation of the respondents, majority of the respondents 139 (36.7%) were housewives. Regarding to educational status of the respondents, high proportion 166 (43.2%) were unable to read and write and the rests were primary school 105 (27.3%) and others . More than half of the respondents were single 261(68%) and the rests were married 101(26.3%) and others and majority of the respondents 1776 (45.3%) had income level between 2501-3500 ETB

Table 1: socio demographic characteristics of patients visiting worabe health center from April 30 to July 30, 2015. (n=384)

variable		frequency	Percent
Sex	**Male**	**182**	**47.4%**
	Female	**202**	**52.6%**
	Total	**384**	**100%**
Age	**<5**	**123**	**32.0%**
	6-15	**99**	**25.8%**
	16-25	**99**	**25.8%**
	26-35	**37**	**9.6%**
	>35	**26**	**6.8%**
	Total	**384**	**100%**
Religion	**Muslim**	**307**	**17.7%**
	Orthodox	**68**	**2.3%**
	Protestant	**9**	**79.9%**
	Catholic	**0**	**0%**
	Others	**0**	**0%**
	Total	**384**	**100%**
Ethnicity	**Silte**	**376**	**97.9%**
	Oromo	**3**	**5%**
	Gurage	**2**	**8%**
	Sidama	**1**	**3%**
	Other	**2**	**5%**
	Total	**384**	**100%**
Educational status	**Unable to read and write**	**166**	**43.2%**
	Reading and writing	**70**	**18.2%**
	Primary	**105**	**27.5%**
	Secondary	**28**	**7.3%**
	Collage/university and above	**15**	**3.9%**
	Total	**384**	**100%**
Occupational status	**Farmer**	**18**	**4.7%**
	Merchant	**30**	**7.8%**
	Student	**142**	**37%**
	Govermnental org	**7**	**1.8%**

	NGO	**1**	**0.3%**
	House wife	**55**	**14.3%**
	Others	**131**	**34.1%**
Marital status	**Single**	**261**	**68.0%**
	Married	**101**	**26.3%**
	Divorced	**8**	**2.1%**
	Widowed	**14**	**3.6%**
	Total	**384**	**100%**
Resident status	**Urban**	**268**	**69.8%**
	Rural	**116**	**30.2%**
	Total	**384**	**100%**
Monthly income	**< or equal to 2000**	**18**	**4.7%**
	2001-3500	**177**	**46.1**
	3501-10000	**174**	**45.3%**
	10001-15000	**15**	**3.9%**
	>15000	**0**	**0%**
	Total	**384**	**100%**

5.2 prevalence of malaria and parasite density

Out of 384 patients who visited worabe health center for medical case, 56(14.6%) are found to be infected with plasmodium parasites as detected by microscopy, among which 42(75%), 11(19.6%), and 3(5.4%) are belong to P.falciparum, P.vivax and mixed infection, respectively.

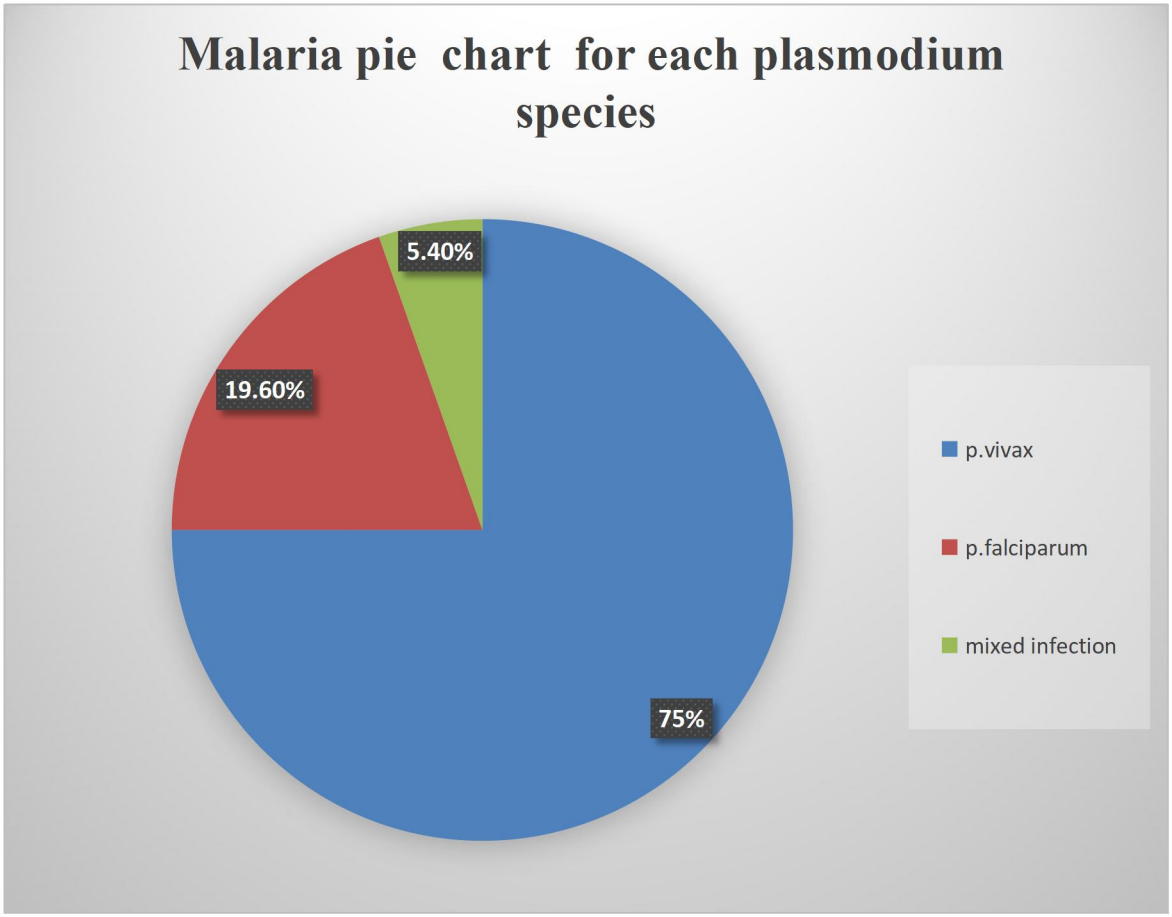

Figure 2: percentage of malaria infection for each population species

5.2.1 prevalence of malaria

5.2.2 Parasite density

Parasite density (count) was determined for all malaria-infected individuals. The majority of them (43%) had moderate parasite density and the remaining 37% had high parasite density and low parasite density(20%).

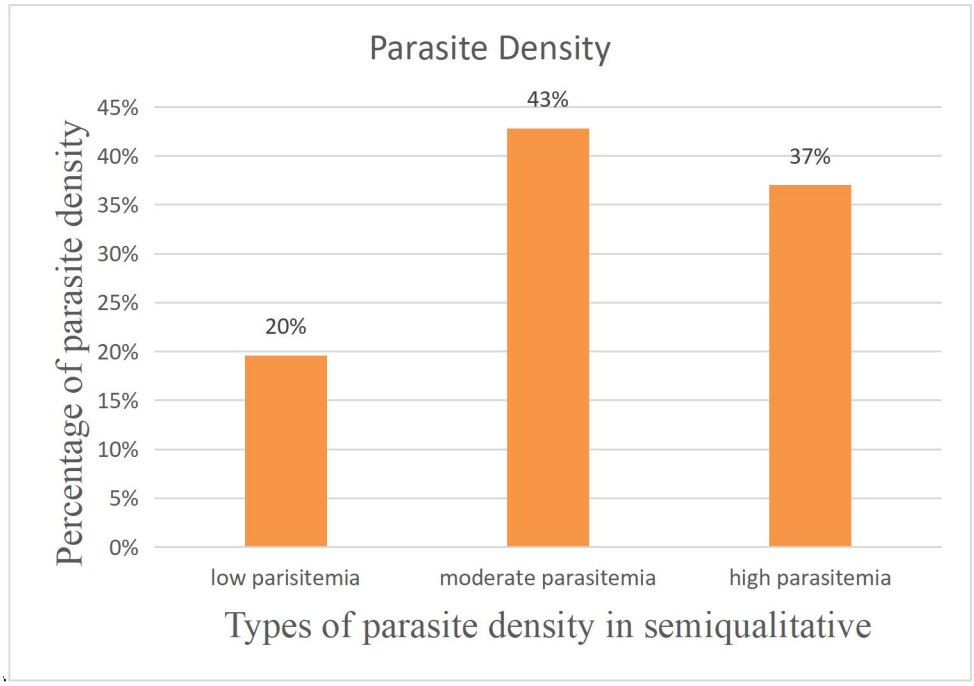

Figure 3: percentage of parasite density in semi-qualitative among study participant of worabe health center, south eastern Ethiopia

5.3 Prevalence of Overall Malaria by Age Groups, Sexes

The prevalence of malaria was found to be the highest among under five age groups(35%) than other age groups, but the difference was not statically significant.

Table 2: prevalance of malaria by age and sex among study participants at werabe health center, southeast Ethiopia (n=384)

Age		Number examined	P falciparum	P vivax	P mixed	Total
<5		123	3 (15%)	17 (85%)	0 (0%)	20 (35%)
6-15		99	6 (31.6%)	12 (63.2%)	1 (5.3%)	19(33.9%)
16-25		99	1(9.1%)	9 (81.9%)	1 (9.1%)	11(19.6%)
26-35		37	1(33.3%)	2 (66.7%)	0 (0%)	3(5.35%)
>35		26	0 (0%)	2 (66.7%)	1 (33.3%)	3(5.35%)
Total		384	11 (19.6%)	42 (75%)	3 (5.4%)	56(100%)
Sex	Male	182	7 (21.8%)	23 (71.8%)	2 (6.25%)	32 (57.1%)
	Female	202	4 (16.7%)	19 (79.1%)	1 (4.2%)	24 (42.9%)
	Total	384	11 (19.6%)	42 (75%)	3 (5.4%)	56 (100%)

5.4 Frequency of ABO Blood Groups among the Study Participants

All patients who suspected malaria infection were also tested for ABO,accordingly; 33.85%, 33.07%, 24.48% and 8.59% were found to be blood types of O, A, B, and AB respectively . Almost all (89.3) of them were RH Positive.

Percentage of the four blood types among the participants

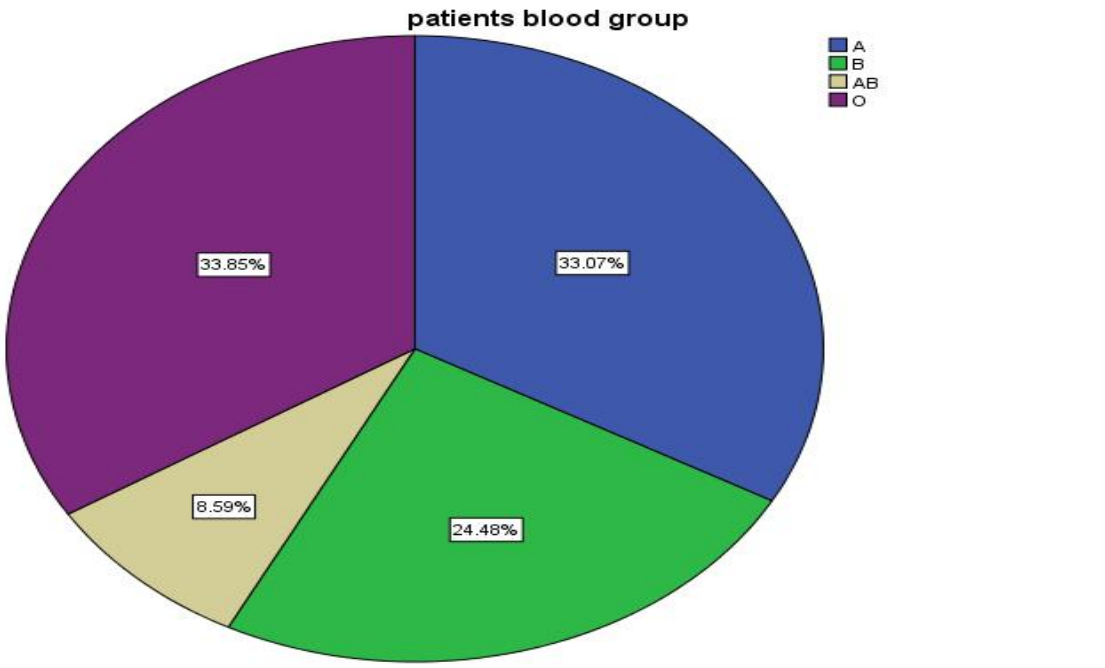

Figure 4: frequency distribution of ABO blood group among participants

5.5 Association of ABO blood groups with malaria infection

384 febrile patients was attended at worabe health center for medical cases, out of those 6.25%,4.1% ,2.86% and 1.3% are plasmodium infected patients with blood groups A, O, B and AB respectively. The left 93.75%, 95.9%, 97.14%, and 98.7% are non-plasmodium infected with blood groups A, O, B, and AB respectively. Greater than half of patients was non plasmodium infected.

Finally, Among plasmodium infected individuals who have blood group A(42.8%),from those the highest percentage was *plasmodim vivax*(79.1%) followed by *p.falciparum*(16.7%) and mixed (4.1%)and also among B blood groups(19.6%) the most prevalence was plasmodium vivax (63.7%) followed by *p-falciparum*(27.4%) proportion of *P.falciparum, P.vivax* and mixed infection of plasmodium with all blood groups was not statistically significant (p-value=0.97)

Table 3: frequency distribution of plasmodium species among the four blood groups (n=384)

Blood group	Identfication of species			Total	
	P.falciparum	P.vivax	mixed infection		X 2 , P -value
A	4(16.7%)	19(79.1%)	1(4.1%)	24(42.8%)	0.369 , 0.975
B	3(27.4%)	7(63.7%)	1(9%)	11(19.6%)	
AB	2(40%)	2(40%)	1(20%)	5(8.9%)	
O	2(12.5%)	14(87.5%)	0(0.0%)	16(28.6%)	
Total	11(19.6 %)	42(75%)	3(5.4%)	56(100%)	

As plasmodium species were identified by using both thick and thin blood film microscopically parasite density also determined for all malaria-infected individuals on thick film. The majority, (42.8%) of patients had moderate parasite density and followed by high parasite density(37.5%) and low parasite density(19.6%). About (50%) of individuals with blood group A and (40%) of individuals with blood group B had high parasite density and this observed difference between blood groups with parasite density was statistically significant (P= 0.012)

Table 4: frequence distribution of parasite density among the four blood groups (n=384)

Parasite density in semi-quantitative	Patient blood group					
	A	B	AB	O	Total	X2,p - value
Low parasitemia	2(8.3%)	4(36.4%)	1(20%)	4(25%)	11(19.2)	0.022, 0.012
Moderate parasitemia	10(41.7)	1(9%)	2(40%)	11(68.8)	24(42.8)	
High parasitemia	12(50%)	6(54.5%)	2(40%)	1(6.3%)	21(37.5)	
Total	24(42.6%)	11(19.4)	5(8.9)	16(28.6%)	56(100%)	

X2: Chi square

5.6. Associated Factors of Malaria Infection

5.6.1 Bivariate Analysis of Malaria with Associated Factors

Bivariate logistic regression was used to examine all possible malaria risk factors by taking(p-value<0.05). The respondents' residence status, utilization of bed net, presence of mosquito breeding site, type of breeding site of mosquito ,house structure were statistically associated with malaria infection in bivariate logistic regressions(p<0.05). However, the respondent;s sex,age,occupational status ,DDT spray status, number of window and ABO blood group were not associated with malaria (P>0.05) .Based on the results of the bivariate analysis, the chances for utilization of bed net to cause malaria infection were about 1.7 times more likely than not utilization of bed nat [COR=1.714 (95%CI= 0.95 -3.08)]. The other socio-demographic variable significantly associated with malaria infection was presence of mosquito breeding site.Absence of of mosquito breeding site were about 0.39 times less likely cause malria infection than presence of mosquito breeding site around there house [COR= 0.39 (95%CI= 0.21-0.75).Individuals who had house structure made with mud had more chances for malaria infection about 1.9 likely than the house made from cement [COR= 1.93 (95%CI 1.04-3.59).

5.6.2 Multivariate Logistic Regression of Associated Factors for Malaria

Potential malaria associated factors that showed P value< 0.25)in the bivariate analysis were selected and entered for multivariate logistic regression to control confounding factors. Eight variables (sex, age group, residence status ,DDT spray status,bed net utilazation ,presense of breeding site ,types of mosquito breeding site and house structure) were selected and entered into the backward stepwise multivariate logistic regression model. But, only one variable(types of mosquito breeding site) appeared in the multivariate model after stepwise selection process. Based on multivarite analysis,the risk of Plasmodium parasite infection who live near stagnant water was 4.3 (AOR=4.388,(95% CI=1.067-18.05) times higher than other mosquito breeding site.

Table 5: Bivariate and multivariante analysis of associated risk factors for malaria infection (n=384)

Variables		Malaria		Bivariante analysis		Multivariate analysis	
		Pos	Neg	COR(95%Cl)	P-value	AOR(95%Cl)	P-value
Sex	Male	32	150	0.63(0.3-1.12)	0.1*	1.05(0.35-6.4)	0.581
	Female	24	178	----	--	--	
Age	<5	20	103	1.78(0.67-4.9)	0.2*	2.7(0.400-19.5)	0.300
	6-15	19	80	2.17(0.81-5.8)	0.1*	11.0(0.8-140.)	0.063
	16-25	8	48	1.46(0.47-4.5)	0.50	11.4(0.46-27.9)	0.136
	26-35	2	40	0.68(0.16-2.9)	0.61	6.7(0.38-118.8)	0.189
	>35(ref)	7	57	------			
Occupational status	Farmer	4	14	1.68(0.5-5.66)	0.4	-	-
	Merchant	2	28	0.42(0.09-1.9)	0.26	-	-
	Student	22	120	1.08(0.55-2.1)	0.81	-	-
	Gov.employe	0	7	0.00	0.99	-	-
	Non.gov	0	1	0.000-	1.00	-	-
	House wife	9	46	1.15(0.48-2.7)	0.74	-	-
	Others(ref)	19	112	-	-	-	-
Resident status	Urban (ref)	31	237	-	-	-	-
	Rural	25	91	2.1(1.17-3.75)	0.01*	3.96(0.48-32.11)	0.197
DDT spray	Yes (ref)	0	3	-	-	--	- -
	No	56	325	0.24(0.04-1.5)	0.13*	0.00	1.00
Bed net utilazation	Yes(ref)	11	149	-	-	-	-
	No	45	178	1.7(0.95-3.08)	0.07*	0.49(0.070-3.49)	0.481
Mosquito breeding site	Yes (ref)	18	52	-	-	-	-
	No	38	276	0.39(0.2-0.75)	0.004*	0.5(0.01-17.28)	0.704
Types of breeding site	Stagnant water	10	12	4.16(1.3-12.9)	0.01*	4.3(1.067-18.05)	0.04**
	Others(ref)	8	39	-	-	-	-
Number of window	1	21	139	1.1(0.24-5.31)	0.87	-	-
	2	28	167	1.25(0.27-5.8)	0.76	-	-
	3 or above(ref)	2	15	-	-	-	--
House structure	Cement (ref	40	185	-	-	-	-
	Mud	16	143	1.9(1.04-3.59)	0.03*	4.6(0.388-55.56)	0.226
Blood groups	A	24	103	1.66(0.8-3.29)	0.14*	1.34 (0.23-7.79)	0.745
	B	11	83	0.94(0.41-2.1)	0.89	0.1 (0.008-2. 36)	0.171
	AB	5	28	1.27(0.43 -3.7)		3.37(0.3-37.23)	0.321
	O	16	114	-	-	-	-

Key * candidate variables at p is less than or equal to 0.25 in bivariatelogistic regression , ** predictor variables in multivariate logistic regresson at p is less than or equal to 0.05,**COR**:Crude Odd Ratio,**CI**:Confidence interval,**AOR**: Adjusted Odd Ratio,**Ref**:Reference

CHAPTER SIX

6. DISCUSSION

In this study,56(14.6%) of malaria patients are found to be infected with plasmodium species. This is comparable with the study conducted in Gonder(14.7%)[1] .But, lower than the study conducted in Dore befano health center,southern Ethiopia(66.2%) [14],Felegeselam health center, Northwestern Ethiopia(50.5%) [18].Moreover, our finding was higher than the study conducted at Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia(8.5%) [20] and urban area in Jimma town (5.2%) [21].The possible reason for the difference could be difference in study design, geographical location (rainfall, temperature and altitude), quality of houses, nature of population, sample size, study period (due to seasonality of malaria in Ethiopia) and the implemented malaria control program in the study area (for instance IRS didn't applied in this year in our study area).

Regarding plasmodium species , highest prevalence was caused by P.Vivax(75%) as compared with P.Falciparum (19.6%) and others .This is contradicted with previous study conducted in northwestern Ethiopia [18] and dore befano health center,southern ethiopia [14], reported that high prevalence of P.falciparum than P.vivax infection,dore befano health center,southern ethiopia.This difference might be due to variation in epidemiological distribution of Plasmodium species in different part of Ethiopia.

In this study the prevalence of malaria was higher in males (57.1%) than females (42.9%).This agrees with previous study conducted in Colombia should the prevalence of malaria higher in males than females[9].This could be due to males send most of his time in outdoor activities than females.so it makes males more exposed to outdoor mosquito bite.

The prevalence of malaria was found to be the highest among under five age group (35.7%) than other age groups this also agrees with study conducted in Dore befano helth center souther ethiopia [14] and contradict with study conducted at health institution in south gonder, the prevalence of malaria was highest among 5-17 age group[1].

The ABO blood group distribution in this study showed group O to be the predominant group occurring in 33.85% of all the patients followed by A (33.07%), B (24.48%), and AB (8.59%) and it's the same with other study.Nearly half (36.4%) of P. falciparum infected individuals were blood group A and about (45.2%) of P. vivax infected individuals were blood group B. The observed difference between plasmodium species *(P.falciparum* and *P. vivax)* and ABO blood groups was not statistically significant(P=0.97), that is malaria infection with *P. falciparum* or *P. vivax* species was not associated with specific ABO blood groups. This finding contradicts with study conducted at Dore befano of southern Ethiopia[14]and at north western Ethiopia[20] ,reporting that malaria infection showed significant association with ABO blood groups . However,the study conducted at federal capital territory of Nigeria[18] and East china[16] reported were agree with our study ,and there was no significant association between ABO blood groups and prevalence of malaria. But, high parasitemia of plasmodium falciparum is observed on blood group B(50%) and A(50%),than AB and O. This may be indicated that blood group A and B showed more susceptibility to severe (complicated)malaria than blood group O. also this agree with research conducted in Nigeria[17], that the protective effect of blood group O was demonstrated with a decreased risk of developing severe malaria (High parasitemia) than other blood groups. It may be due to sequestration and rosatte formation of blood group A and B.The observed difference between blood groups with parasite density was statistically significant. Based on multivarite analysis,the risk of Plasmodium parasite infection who live near to stagnant water was 4.3 times higher than other mosquito breeding site like pond, ditches and etc.

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

The overall prevalence of malaria was 14.6%.andPlasmodium vivax is the dominant species in the study site .Moderately high prevalence of malaria infection was found among under five children. In this study blood group O is the predominant than other ABO blood groups. This study revealed that ABO blood groups have no association with malaria infection caused by either P.falciparum or P. vivax species.. Individuals with blood group A had increased risk of developing severe (high parasitemia) 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.In this study educational status, and occupational status of study subjects had no association with malaria infection. Similarly, Sex, age, DDT spraying ,housing structure ,bed net utilization and mosquito breeding site was not statistically associated. The only statically significant variable is types of mosquito breeding site

7.2 Recommendation

- ✧ **For zonal and werada health office of worabe town**

We would like to recommend zonal health office and woreda health office of worabe town:

- ✓ In order to reduce prevalence of malaria by cooperative working and teaching communities about the effect of malaria and
- ✓ Doing activities like removing stagnant water around the house, using mosquito sprays and bed nets.

- ✧ **For further investigators(Researchers)**

We would like to recommend:

- ✓ 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
- ✓ Further study on the bed net utilization rate for malaria risk groups, especially under five year age should be studied

7.3 Limitations and strength of the study

Limitation of the study

- ✓ Time of data collection affected the prevalence due to seasonal variation of malaria transmission.
- ✓ The study was not included qualitative method, particularly observation of housing conditions and bed net utilization status.
- ✓ Since we used a Convenient sampling technique, we cannot make infer to the general population of of worabe town.

Strength of the study

- ✓ Willingness of staffs of the health center in providing necessary information and supporting participation during data collection
- ✓ Good respond and willingness of the study subject to participate in the study

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ANNEX I
QUESTIONNAIRE

Consent and information sheet

Questionnaire for study of malaria prevalance and its association with ABO blood groups among study participants at worabe health center

Dear respondent

My name is_____ I am currently a member of data collectors from a researcher which is conducted by Wolkite university department of Medical laboratory.

Introduction

The objective of this study is to determine the prevalence of malaria infection and its association within ABO blood group among acute febrile patient attending Worabe Health center. We received approval letter from university with permission of research committee and the questioner include socio- demographic factor.

You will not write your name on the questionnaire and your individual responses will be not reported. We would also like to assure you about the confidentiality of your information that it will be locked with key till entered into a computer with password than the questionnaire paper will be burned. Also, the information will be used for this research only. The study does not cause any harm other than expensing you a few minutes to answer the questions

Code No_____Date_____

Part-ISocio-demographic characteristics

1. Gender a. Male B. female

2. Age-------- years

3. Religion a. Orthodox b. Protestant

c.Muslim d. Other specify---------

4. Ethnicity a. Amhara c. Oromo

b. Gurage d. Other specify------

5. Marital status

a. Single b. Married

divorced d. Widowed

6. Educational status

a. Illiterate b. Reading and writing

c. Primary school d. Secondary school

e.College/Diploma f.university/Degree g.Postgraduate

7. Occupational status of patient

a. farmer b.merchent

c.student d. governmental employe

e.Non.gov.employe f. House wife g.others

8 If the patient is not married,

A.Occupation status of patient's Father

a. farmer b.merchent

c.student d. governmental employe

e.Non.gov.employe f. House wife g.other

B.Occupation status of patient's Mother

a. farmer b.merchent

c.student d. governmental employe

e.Non.gov.employe f. House wife g.others

9. Resident status of patient 1.Urban 2. Rural

10. Average monthly income----------(birr)

part-II Assessing associated factors .

11.Do you have mosquito bed net in your house 1.Yes 2.NO

12.If yes,

13.Do you use it ?

A.YES B. NO

14.Do you regularly spray DDT in your house?

A. Yes B. No

15. If yes, how often? ---------- A.1time B.2 times C.3times D.4times

16.Is there mosquito breeding site around your house area? A. Yes B. No

17. If yes, of the following which one?

A.Stagnant water B. Pond C. Ditches D. Others(specify)

18. Does your house have a hole? A) yes B) No

19.what is your house made from? A) cement B) mud C) other

20.how many windows are there in your house? A) 1 B) 2 C) 3 D) More than 3

21.Had you take any drug before you come to here A. Yes B. NO

21 • If yes, which one?

A. Anti-malaria B. Anti-pain C. Other(specify)----------

part- III. Results of laboratory investigation

22.The result of blood film

A.No H/P is seen

B.Plasmodium spp is seen

23. If so,Identify plasmodium species

a.P. falciparum b.P. vivax

c. Both p. falciparum and p. vivax d. Other

24 Parasite density ……Parasite per microlitre

A.lowdparasite B.moderateparasite C.highparasite D.veryhighparasite

25 Blood group ----------A.A B.B C.AB D.O

26.Rhesus blood group A.Postive B.negative

ANNEX II

sample collection and examination procedure

I) Sample collection procedure for detection of malaria

1. Assemble the necessary materials and equipment.
2. Identify the patient and allow him/her to sit properly and give the finger comfortable.
3. Prepare the finger by swabbing cotton moistened with 70% alcohol.(for infant I use heel puncture).
4. Collect capillary blood by using sterile disposable lancet.
5. Immediately, thin film will be spread on a grease free, frosted end labeled slide using smooth edged slide spreader and the thick blood film will be also prepared on the same slide.
6. Thin film will then fixed with methanol (not thick film), then dry.
7. The blood film will be stained with Giemsa stain for 10 minutes.
8. Then, the blood film will be examined by laboratory technologist using an oil immersion microscope objective (100x).
9. Then, the result will noted on the data collection format.

II) Sample collection procedure of ABO blood group test (by using direct method agglutination test).

1. Assembly the necessary materials and equipment.
2. Identify the patient and allow him/her to sit properly and give finger comfortably, I use heel for infants.
3. Prepare the finger or heel by swabbing cotton moistened with 70% alcohol. Then dry it.
4. Collect capillary blood by using sterile disposable lancet.
5. Immediately, transfer two drops of whole blood placed in two different places a grease free clean glass slide on which few drops of antisera for blood group A & B will be applied.
6. The blood cells and the antigen mixed with separate applicator stick.
7. The slide will be then titled to detect for agglutination and the result recorded accordingly.

N.B a normal and abnormal quality control sample (assayed or commercially manufactured) should be analyzed with patient sample to detect analytical errors.

ANNEX III
Materials and reagents

Materials

- ✓ Microscopic slides
- ✓ Pencil
- ✓ Microscope
- ✓ Cotton swab
- ✓ Staining rack or staining jar
- ✓ Glove
- ✓ Pen
- ✓ Syringe or lancet
- ✓ Waste disposal …

Reagent

- ✓ Methyl alcohol (Methanol)
- ✓ geimsa stain (10%)
- ✓ 70% Alcohol
- ✓ Oil immersion
- ✓ Tap water
- ✓ Anti-A and anti –B antisera
- ✓ Anti –D antibody

Cleaning agent

- ✓ Bleach
- ✓ Soap

ANEX IIII Declaration Sheet

Declaration

The undersigned declares that this thesis complies with the university's regulations and meets the accepted standards concerning originality and quality. Principal investigators also agrees to take responsibility for the research project's scientific, ethical, and technical conduct and the provision of required progress reports.

BSc candidate students name:

ABEBE TALEMA Signature: _____Date of submission: 17/12/2015

BEKELE MERSHA Signature: _____Date of submission: 17/12/2015

HAYAT MUSTEFA Signature: _____Date of submission: 17/12/2015

APPROVAL OF THE ADVISORS

This research paper approved by University advisors:

1. Name of 1st advisor: Mr.Lema Workineh (BSc, MSc, Assistant professor)

Signature: _____ Date of submission:17/12/2015

2. Name of 2nd advisor: Mr. Dereje Abebe(BSc, MSc)

Signature: _____ Date of submission:17/12/2015

Examiner

Name: _____Signature: _____Date_____/_____/_____

Name: _____ Signature: _____Date_____/____/_____

Name of School head: _____

Signature: _____ Date of submission: 17/12/2015