THERAPEUTIC EFFICACY OF CHLOROQUINE FOR TREATMENT OF *PLASMODIUM VIVAX* MALARIA AMONG OUTPATIENTS AT HOSSANA HEALTH CENTER, SOUTHERN ETHIOPIA

BY

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A THESIS SUBMITTED TO JIMMA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF DEGREE OF MASTERS OF SCIENCE IN MEDICAL PARASITOLOGY

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JIMMA, ETHIOPIA

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Abstract

Background: *Plasmodium vivax accounts for about 44 % of all malaria infection in Ethiopia. Chloroquine is the first line treatment of Plasmodium vivax malaria in Ethiopia. However, chloroquine is first line drug used to treat P.vivax malaria in Ethiopia, some reports indicating the emergence of chloroquine resistant vivax malaria in different parts of the country. Chloroquine resistant Plasmodium vivax has been emerging in different parts of the world, and posing both health and economic impact especially in developing countries.*

Objective*: To determine therapeutic efficacy of chloroquine for the treatment of Plasmodium vivax malaria among outpatients in Hossana Health centre, Southern Ethiopia.*

Methods: *A one arm 28 days in vivo drug efficacy study was conducted at Hossana Health Centre from April 5 to June 25/2014. Convenient sampling technique was used to enrol 63 patients aged between 4 and 59 years with microscopically confirmed Plasmodium vivax. All patients were treated with chloroquine 25 mg/kg for three days. Recurrence of parasitaemia and clinical conditions of patients were assessed on day 1, 2, 3, 7, 14, 21, and 28 during the 28 day follow-up period. Haemoglobin level was determined on day 0, day 28 and on day of recurrence of parasitaemia by using portable spectrophotometer.*

Results: *From 63 patients included in the study, 60 (95.2%) completed their 28 days follow up, 3 patients excluded from the study;1 patient due to vomiting of the second dose of drug, 1 patient due to Plasmodium falciparum infection and 1 patient lost to follow up the study. During enrolment, 35(53.3 %) had a history of fever and 28(46.7 %) had documented fever. The geometric mean of parasite density on day of enrolment was 3472 parasites/µl. Among these, 2 patients had recurrent parasitaemia within the 28 day follow up.*

Conclusion*: Chloroquine was found to be efficacious (96.7%) except 2 treatment failures detected in Hossana Health Centre Southern Ethiopia This failure is most likely late parasitological failure.*

Recommendation*: Regular monitoring of the pattern of resistance to chloroquine is needed in Plasmodium vivax malaria endemic areas of the country to take measures rapidly and effectively to control the possible spread of drug resistance.*

Key words*: Plasmodium vivax, Chloroquine resistance, therapeutic efficacy, Ethiopia*

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List of Abbreviations and Acronyms

CDC Center for Disease Control

CRPV Chloroquine Resistant *Plasmodium Vivax*

DACA Drug Administration and control Authority

ETF Early Treatment Failure

FMOH Federal Ministry Of Health

Hb Haemoglobin

LCF Late Clinical Failure

LPF Late Parasitological Failure

Mg Milligrams

MOH Ministry Of Health

Ng Nanogram

PCR Polymerase Chain Reaction

PCT Parasite Clearance Time

SNNPR South Nations Nationalities and Peoples Region

SP Sulphadoxinepyramethamine

SPSS Statistical Package for Social Science

WBC White Blood Cells

WHO World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Back ground

Malaria is caused by protozoan parasites of the genus *Plasmodium*. There are four species that infect human ,which are *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale* (Okwa, 2012, Ridley, 2012). Occasionally, human beings can be infected either naturally or accidentally by several simian species such as *Plasmodium cynomolgi, Plasmodium cynomolgi bastianelli, Plasmodium brasilianum and Plasmodium knowlesi. P.knowlesi* emerged as an important cause of human malaria in South-East Asia, especially the Malaysian Borneo since 2004 (Antinori *et al*., 2012).

Globally, an estimated 3.3 billion people were at risk of malaria in 2011, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria: approximately 80% of cases and 90% of deaths are estimated to occur in the WHO African Region, with children under five and pregnant women most severely affected (WHO, 2012, CDC, 2012). There were an estimated 627 000 malaria deaths worldwide in 2012. It is estimated that 90% of deaths in 2012 were in the African Region, followed by the South-East Asia Region (7%) and Eastern Mediterranean Region (3%). About 482 000 malaria deaths were estimated to occur in children under 5 of age, or 77% of the global total. An estimated 462 000 of deaths occurred in children under 5 years of age in the African Region (WHO, 2013).

Malaria is a leading public health problem in Ethiopia. It is estimated that about 75% of the total area of the country and 65% of the population is at risk of infection (MOH, 2004, CDC, 2012). *P.falciparum* and *P.vivax* are the main two species accounting for 56 % and 44% of malaria cases respectively (WHO, 2013). In 2009/2010, malaria was the first cause of outpatient visits, health facility admissions and in-patient deaths, accounting for 12% of out-patient visits and 9.9% of admissions. Malaria is one of the top ten causes of inpatient deaths among children less than five years of age (CDC, 2013a).

P. falciparum and *P. malariae* occur worldwide. But, *P. ovale* occurs in Africa and in foci within Asia and Oceania, and is often present with other Plasmodium species as a mixed infection (Mandell *et al*., 2010). On the other hand, *P. vivax* is present throughout the tropics with low rate of infection in western and central sub Saharan Africa due to duffy coat antigen

(Culleton *et al*., 2008, Abdallah *et al*., 2012, Baird, 2009). According to recent estimates 40% of the world's population (2.6 billion people) is at risk of *P.vivax* transmission with between 130-435 million clinical episodes of *vivax* malaria each year (Antinori *et al*., 2012). *P. vivax* accounts for more than 50% of all malaria cases outside Africa, with 80-90% occurring in the Middle East, Asia and the Western Pacific, and 10-20% in Central and South America (Price *et al*., 2007 , Guerra *et al*., 2010).

Malaria parasites are transmitted by the bite of an infected female Anopheles mosquito. Sporozoites contained in the saliva of the mosquito are inoculated into the blood of a human host when the mosquito takes a blood meal. Infection can also occur by transfusion of infected donor blood, by injection through the use of needles and syringes contaminated with infected blood, and very occasionally congenitally, usually when a mother is non-immune (Cheesbrough, 2009).

P. vivax infections have been associated with mild symptoms, such as fever, headache, fatigue, chills, and musculoskeletal pain, and, in particular, paroxysms. Recently, however, severe complications, including renal failure, jaundice, acute respiratory distress syndrome, cerebral malaria, anemia, hyperparasitemia, thrombocytopenia, pulmonary edema, splenic rupture, and death, have been reported in exclusive association with *P. vivax* (Baird, 2007).

The first line drug for *P. vivax* is chloroquine 150 mg base tablet or chloroquine syrup 50 mg base. The ideal chloroquine dose is 10 mg base/kg immediately (Day 1) followed by 10 mg base/kg at 24 hours (Day 2) and 5mg base/kg at 48 hours (Day 3) for a total dose of 25 mg chloroquine base/kg over three days (FMOH, 2012). However, chloroquine is first line drug used to treat *P.vivax* malaria in Ethiopia, some reports indicating the emergence of chloroquine resistant vivax malaria in different parts of the country.

In Ethiopia chloroquine was the first line antimalarial drug used to treat falciparum malaria for decades. But, chloroquine resistance to *P.falciparum* emerged and first documented in 1986 from boarder areas of Somalia, Kenya and Sudan, where population movements are intense in both directions (Teklehaimanot, 1986). In 1998, intense resistance of the parasite to chloroquine forces change to sulphadoxinepyramethamine (SP).Faster drop in therapeutic efficacy of SP for the treatment of uncomplicated falciparum malaria enforced the adoption of artemether-lumefantrine as a first line treatment in 2004. Since 2004, artemether-lumefantrine

used as a first line treatment against uncomplicated *P.falciparum* malaria in Ethiopia (MOH, 2004).

Anti-malarial drug resistance is the ability of a parasite to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject. Many factors can contribute to treatment failure including incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or inconsistent absorption, and misdiagnosis. Probably all of these factors, while causing treatment failure in the individual, may also contribute to the development and intensification of true drug resistance through increasing the likelihood of exposure of parasites to suboptimal drug levels (WHO, 2001).

1.2 Statement of the problem

Malaria is a major public health problem and cause of much suffering and premature death in the poorer areas of tropical Africa, Asia and Latin America and occurs in 110 countries with about 40% of the world's population at risk (WHO, 2013).

Approximately 52 million people live in malaria risk areas in Ethiopia. Malaria is mainly seasonal with unstable transmission in the highland border areas and of relatively longer transmission duration in lowland areas, river basins and valleys. Historically, there have been an estimated 10 million clinical malaria cases annually. Rates of morbidity and mortality increase dramatically (i.e. 3-5 fold) during epidemics. Since 2006, however, cases have reduced substantially because of malaria control programs (FMOH, 2012).

Therefore, the control strategies include chemotherapy, insecticides and use of mosquito bed net (WHO, 2010b). The emergence and rapid spread of resistant parasites to antimalarial drugs and vector to insecticides respectively has worsened the problem of control (WHO, 2001). Chloroquine is the most common and cheapest antimalarial drugs available at the peripheral level (sub health post and health post without laboratory facilities). It is used for treatment of laboratory confirmed *P. vivax* and clinical malarial cases (DACA, 2010b). However, resistance to this drug has alarming proportion in most countries affected by *P.vivax* malaria Ethiopia (Tulu *et al*., 1996, Teka *et al*., 2008, Ketema *et al*., 2009, Ketema *et al*., 2011), Madagascar (Barnadas *et al*., 2008),Myanmar (Guthmann *et al*., 2008),Indonesia (David *et al*., 1998). In such condition, urgent assessment is needed for obtaining the information to develop or modify the treatment policies (WHO, 2001, WHO, 2009).

High-level of chloroquine resistance has been well documented on the northern part of the island of New Guinea and in Sumatra, Indonesia, and there have been sporadic reports from other geographic locations (WHO, 2007).

The first treatment of humans with chloroquine occurred in 1936 four syphilis patients in Dusseldorf, Germany. It has been the first-line therapy for *vivax* malaria since 1946. Emerging resistance to chloroquine by *P. vivax* threatens the health of the hundreds of millions of peoples. *P. falciparum* developed resistance to chloroquine in the 1950s and it occurs globally. Resistance by *P. vivax* was unknown until 1989, when Australians return from Papua New

Guinea failed routine treatment. Subsequent reports confirmed the emergence of chloroquine resistant *P. vivax* malaria from Indonesia (David *et al*., 1998), Myanmar (Guthmann *et al*., 2008) and India (Baird, 2004).

The World Health Organization (WHO) recommended that drug efficacy be regularly assessed. Failure to detect the emergence of anti-malarial drug resistance, could lead to a drug-resistant malaria epidemic, which would have major public health and economic consequences for an area, province and country (WHO, 2009, WHO, 2001, WHO, 2011).Therefore monitoring of drug resistance is essential for timely changes to treatment policy, which should be initiated when the treatment failure rate exceeds 10% at the end of follow-up (WHO, 2009). However a decision to change treatment policy may be influenced by a number of additional factors, including the prevalence and geographical distribution of reported treatment failures, health service provider and/or patient dissatisfaction with the treatment, the political and economic context, and the availability of affordable alternatives to the commonly used treatment (MOH, 2006).

Chloroquine is the preferable drug for treating acute malaria in endemic settings. It is cheap, and effective against *P.vivax,* deliverable over a brief period in few doses, and safe even for pregnant women and small children and it has few side effects (Baird, 2009)**.**Thus chloroquine remains an effective choice of treatment for *P. vivax* infections except for infections acquired in Papua New Guinea or Indonesia. Persons acquiring *P. vivax* infections in Papua New Guinea or Indonesia should initially be treated with a regimen recommended for chloroquine resistant *P. vivax* infections. The three treatment regimens for chloroquine resistant *P. vivax* infections are quinine sulfate plus doxycycline or tetracycline, or, Atovaquone-proguanil, or mefloquine. These three treatment options are equally recommended (CDC, 2013b).

Emergence and spread of chloroquine resistant *P. vivax* malaria in different countries is becoming a public health problem which requires regular monitoring to control its spread(WHO, 2013).There were attempts to control the spread of chloroquine resistance *P.falciparum,* that we learn from Malawi, where chloroquine was withdrawn from the market for 12 years and then re-introduced, showed efficacy from less than 50% to 90% (Laufer *et al*., 2006).There are few reports of chloroquine resistant *P.vivax* in different parts of Ethiopia (Teka *et al*., 2008, Ketema *et al*., 2009, Ketema *et al*., 2011). Therefore, these reports are a good

indicator of the emergence and spread of chloroquine resistant *P. vivax* strains in malaria endemic area of the country. However, more studies are needed on the degree of resistance of *P.vivax* in other malaria endemic areas of the country to understand its distribution. To our knowledge, there is no information on therapeutic efficacy of chloroquine for treatment of *P. vivax* malaria among outpatients in Hossana Health Centre. Thus the present study was aimed at assessing therapeutic efficacy of chloroquine for the treatment of *P. vivax* malaria among outpatients in the study area.

CHAPTER TWO

LITERATURE REVIEW

The first chloroquine resistance of *P. vivax* were reported from Papua New Guinea in 1989 (Rieckmann *et al*., 1989).Chloroquine resistant *P. vivax* has been emerging in different parts of the world. *P. vivax* resistance to chloroquine is common in many parts of the world (Baird, 2004). There are few studies conducted in Africa. In study conducted in Madagascar showed recurrence of parasite in 10 patients from a total of 105. However 10 patients showed recurrence of the parasite, only 5 of them were confirmed as treatment failure after Polymerase Chain reaction (PCR) performed which revealed a failure rate of 5.1% and blood concentration were not done in patients showed recurrence (Barnadas *et al*., 2008).

Study in Ethiopia for the first time on chloroquine efficacy to *vivax* malaria was conducted in Debrezeit. A total of 225 patients were included and followed in this study for seven days, 2 % failed to respond to treatment (Tulu *et al*., 1996). After 12 years, another 28 days therapeutic efficacy study was conducted in same area and 87 patients were involved. In this study, 4 (4.6%) treatment failure was observed during the follow up period. The observed treatment failure was revealed in children below seven years of age. The study showed increment of chloroquine resistance from previous study in 7 days efficacy test before a decade in that similar area (Teka *et al*., 2008).

Also another 28 days efficacy study was conducted in Jimma zone serbo town western Ethiopia which showed a 3.6 % prevalence of chloroquine resistance *P. vivax* malaria (Ketema *et al*., 2009). Recently 28 days chloroquine efficacy study was done in Halaba district of south Ethiopia which showed 13 % prevalence indicating the rise of chloroquine resistance in Ethiopia when it is compared with previous studies (Ketema *et al*., 2011). In Turkey Sanliurfa province therapeutic failure rate of 9.5% was detected in 28 day follow up study (Dilmaec *et al*., 2010).

A study conducted in Indonesia in1998 showed resistance in 12 (23%) from 52 patients between day 7 and 28 (David *et al*., 1998). The highest rate of therapeutic failure which is (84%) was also reported in 2003 in the North Eastern coast of Indonesian Papua (Sumawinata *et al*., 2003);and 56% resistance was reported from Eastern Indonesia in 2009 (Sutanto *et al*.,

2009). The lowest resistance (0.8%) on therapeutic efficacy study was reported from Dawei, Southern Myanmar in 2008 (Guthmann *et al*., 2008). In study conducted in India among patients followed for 42 days, only one Late Clinical Failure (LCF)was found, but the study could not specify the case as either relapse or reinfection because they could not amplify the parasite DNA (Ganguly *et al*., 2012).

On the other hand, the findings of studies conducted in Iran and neighbouring countries like Afghanistan and Pakistan showed susceptibility of *P. vivax* to chloroquine in the region (Heidari *et al*., 2011, Nateghpour *et al*., 2007, Heidari *et al*., 2012). Also in 28 days study made in Colombia from 44 *P.vivax* infected patients treated with standard dose of chloroquine, no therapeutic failure were revealed (Castillo *et al*., 2002). Also in another study no treatment failure was recorded in 14 days of in vivo drug efficacy test carried out in 2005 in Kanchanpur district of India in all 84 *P.vivax* cases, which indicate the susceptibility of chloroquine at that district (Pant *et al*., 2005).

The first chloroquine resistant vivax malaria in an infant was reported from India. A 4-month old girl presented with fever of four days. Her peripheral smear showed ring forms of *P. vivax* and treated with chloroquine but there was no parasitological and clinical cure 3 days after treatment (Shah, 2008). Some of treatment failures recorded in the previous studies have occurred in young children (Teka *et al*., 2008, Ketema *et al*., 2011).

There are studies from different Latin America countries indicate the presence of chloroquine-resistant *P. vivax* malaria. In Colombia a 28-day follow-up study showed treatment failure of 11.1 % in 2001 (Soto *et al*., 2001). In Guyana, three patients who acquired *vivax* malaria treated with standard chloroquine therapy failed even with therapeutic blood levels. Two of the patients had recurrences of *P.vivax* malaria 6 — 8 weeks after receiving directly observed therapy. These cases confirm the presence of chloroquine-resistant *P. vivax* in Guyana (Elizabeth *et al*., 1996). A 28-day in vivo test was conducted in Manaus, Brazil, to assess the efficacy of standard supervised therapy. Among the 109 volunteers who completed the in vivo test, 19 had positive blood smears within the 28-day follow-up and finally chloroquine resistance was confirmed in 11 (10.1%) of study participants (Simoes *et al*., 2007) .

Anaemia is a major problem of malaria infection. Treatment with suitable drug will improve the patients' haemoglobin level. However, in patients with treatment failure, absence of Hb recovery on day of recurrence may indicate presence of sub-microscopic parasitaemia. In a study conducted in Serbo, there was recovery of haemoglobin among study participants with adequate clinical and parasitological responses (Ketema *et al*., 2009).

According to WHO, clinical and parasitological cure is the main target of in vivo therapeutic efficacy study. In a study conducted in Halaba district, southern Ethiopia ,29% of the study participants were with a complaints of malaria symptoms such as headache, fever, chilling, and joint pain, during the follow-up period even though there were not confirmed parasitaemia (Ketema *et al*., 2011). In a study conducted in Serbo none of the patients with treatment failures had complained of malaria symptom among three patients those showed recurrent parasitaemia within the 28 days follow-up. In all patients with treatment failure, parasite count increased from day of admission to day of parasite recurrence without complaints of malaria symptom except for the recurrence of parasitaemia (Ketema *et al*., 2009).

2.1 Significance of the study

Chloroquine is the first line treatment of *P. vivax* malaria in Ethiopia. Few reports are indicating the emergence and spread of chloroquine resistant *P. vivax* strains in malaria endemic areas of Ethiopia. Therefore, this study helps providing the degree of distribution of the chloroquine resistance pattern of *P.vivax* in the study area. Thus, the finding of this study will be helpful for the responsible authorities to take appropriate intervention measures and control further expansion of chloroquine resistance.

CHAPTER THREE

OBJECTIVES

3.1. General objective

- To determine the therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria among out patients in Hossana Health Centre Southern Ethiopia.

3.2. Specific objectives

- To determine the outcome of treatment with chloroquine on clinical and parasitological responses by proportion of ETF, LCF, LPF and ACPR
- To assess the outcome of treatment with chloroquine on parasite and fever clearance time
- To assess rate of recurrence of parasitaemia in patients on each visit during the follow-up period
- To measure the haemoglobin level of patients on day 0, day 28 and on day of recurrence of parasitaemia.

CHAPTER FOUR

METHODS AND MATERIALS

4.1. Study area and period

The study was conducted from April 6 to June 25, 2014 in Hadiya Zone Hossana Town. The Town is located at the margin of Great Ethiopian rift valley in the North Western part of South Nation's Nationalities and Peoples Region (SNNPR). Hossana is capital of Hadiya Zone which is located 230 Km south of Addis Ababa, and 145 Km from Hawassa. The town lies at a longitude of 10°060N39°590E and latitude of 10.1°N39.983°E. The town has a total population of 104,208. The area has short rainy season from March to May and receives high rainfall during the main rainy season (June to September) and is characterized by markedly unstable seasonal malaria. Malaria is one of the main diseases in the town and surrounding that is reported throughout the year (Mulugeta, 2013).

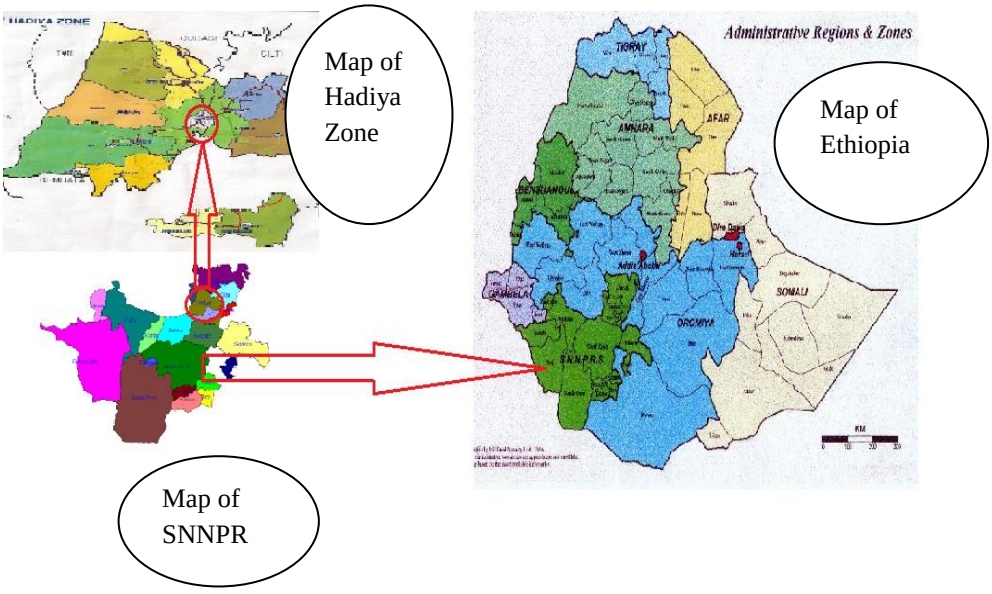

Figure 1: Map of the study area (Mulugeta, 2013)

4.2. Study design

This study was one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment for *P.vivax* malaria. Patient with *P.vivax* infection, who meets the study criteria were enrolled, treated with chloroquine and followed for 28 days. The follow-up included a fixed schedule (1^{st}, 2^{nd}, 3^{rd}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days) of check-up visits and corresponding clinical and laboratory examinations. The proportion of patients with therapeutic failure during the follow-up period was used to estimate the efficacy of chloroquine (WHO, 2009).

4.3. Population

4.3.1 Source population

Clinically malaria suspected individuals with fever or history of fever and seeking treatment at Hossana Health Centre during the study period.

4.3. 2 Study population

Those patients who have confirmed *P. vivax* mono-infection on thick and thin blood film preparations and fulfilled the inclusion criteria (WHO, 2009) and seeking treatment at Hossana Health Centre during the study period.

4.4. Sample size and Sampling techniques

4.4.1 Sample size

The required sample size for this study was calculated based on the prevalence of 3.6% treatment failure in study conducted at Serbo (Ketema *et al.,* 2009) , 5% precision, 95% confidence level, using single population proportion formula (Daniel and W, 1995).

$$n = \frac{Z^2 \times p(1-p)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.036\ (1-0.036)}{(0.05)^2} \qquad n = \mathbf{53}$$

Where, n = number of samples

P = prevalence of resistance in study conducted at Serbo (3.6%) (Ketema *et al.,* 2009)

z = confidence interval (95%), d = precision (5%)

Assuming an additional 20 % (10 patients) loss to follow up rate and withdrawal of consent during the study. Therefore, the final sample size for this study was 63 (53+10).

4.4.2 Sampling technique

Sixty three *P.vivax* mono infected patients were selected by convenient sampling technique i.e. patients who have *P.vivax* infection were selected consecutively.

4.5. Inclusion and Exclusion Criteria

4.5.1 Inclusion Criteria

(WHO, 2009, WHO, 2001)

- Age over 6 months
- Mono-infection with *P. vivax* detected by microscopy
- Asexual parasite count > 250/µl
- Axillary temperature ≥ 37.5 °C or history of fever during the 48 hours before recruitment
- Ability to swallow oral medication
- Willingness to comply with the study for the duration of the study
- Informed consent from the patient and from a parent or guardian in the case of children

4.5.2 Exclusion Criteria

(WHO, 2009, WHO, 2001)

- *Vivax* malaria requiring hospitalization
- Severe malnutrition
- Febrile condition due to diseases other than malaria
- Regular medication, which might interfere with antimalarial pharmacokinetics
- Pregnancy and breastfeeding

4.6. Data collection methods

4.6.1 Questionnaire

Questionnaire was used to gather general information such as socio-demographic information from study participants. The questionnaires were administrated by Senior Laboratory Technologists and checked by principal investigator for completeness (Annex III).

4.6.2 Physical examination

Physical examination was done by Health Officers for all study participants such as the axillary temperature, weight, and clinical condition was assessed during follow up.

4.6.3 Laboratory examinations

4.6.3.1 Parasite detection

Capillary blood was collected from each study participant; thick and thin blood films were made in each follow up days (Days 1, 2, 3, 7, 14, 21 and 28) and stained using 10 % Giemsa staining for 10 minutes and air dried the film and examined under microscope by using 100x oil immersion field and report was recorded on the laboratory request (Annex IV and V).

4.6.3.2 Parasite count

The thick blood smear was used to count the numbers of asexual parasites and White Blood Cells (WBCs) in a limited number of microscopic fields. *P. vivax* asexual stages counted against 200 WBCs. Parasitemia was determined according to formula below (WHO, 2009).

$$\text{Parasite density (per µl of blood)} = \frac{\text{WBC count (8000) X Number of parasites counted}}{\text{Number of WBC counted (200)}}$$

4.6.3.3 Haemoglobin Measurement

Haemoglobin was measured on day 0 and 28 during follow-up for each study participants and on day of recurrence of parasitaemia. Finger prick was taken and read by portable spectrophotometer (HemoCue Hb 301 system, Sweden) (Annex VI). Anaemia was defined according to (WHO, 2011b) categorization (Annex VII).

4.6.3.4 Pregnancy test

Urine of all women aged 13–49 was screened for pregnancy by Strip test, (Manufacturer PR China, Manufacturing date 2012/11, Expiration date 2014/11, Batch number W00121125.2) was used and those testing positive were excluded from the study.

4.7. Variables

4.7.1 Dependent variable

- Therapeutic efficacy of chloroquine

4.7.2 Independent variables

- Sex
- Age
- Occupation
- Educational status

4.8. Treatment procedure

Based on the treatment guidelines of the Federal Ministry of Health of Ethiopia (FMOH 2012) the patients were treated with a 25 mg/kg chloroquine (Manufacturer Addis Pharmaceuticals factory, Adigrat, batch number 9461, manufactured date 02/2011 and date of expiration 02/2016), administered for 3 consecutive days (10 mg/kg, 10 mg/kg, and 5 mg/kg on day 0, 1, 2 respectively) (DACA, 2010b, DACA, 2010a, FMOH, 2012). All doses administered under direct observation. Study subjects checked for vomiting for 30 minutes after intake of the drug; those who vomit were re-treated with the same dose. Subjects who vomit twice are excluded from the study. The study participants advised not to take other drugs, except for patients with axillary temperature > 37.5°C treated with paracetamol (10 mg/kg). Patients were encouraged to come back to the Health centre if they feel sick at any time during the follow-up period for clinical and parasitological examination. In particular, parents or guardians were instructed to bring children to the centre at any time if they show any sign of danger (unable to drink or breastfeed, vomiting, presenting with convulsions, lethargic or unconscious, unable to sit or stand, presenting with difficult breathing), if they are still sick or if there is any cause for worry (WHO, 2009).

4.9. Follow-up

Patients who meet all the enrolment criteria were given a personal identification number and received treatment only after the study has been fully explained to them and they have willingly provided informed consent. Person who decides not to participate in the study was examined, treated and followed-up by the health facility staff. A successive monitoring of parasitological and clinical responses was made for 28 days with each patient. The day a patient is enrolled and receives the first dose of medicine is designated as ‘Day 0’. Patients were informed to return for follow-up on Days 1, 2, 3, 7, 14, 21 and 28. Thick and thin blood smears were prepared and

examined for checking parasite clearance and/or recurrence of parasitaemia at all follow-up visits. Haemoglobin measurement was made on day 0 and 28 during follow-up and on day of recurrence of parasitaemia. Any patient who didn't come to the Health centre on the day of appointment was traced at his/her home and was assisted by the health extension workers to complete the follow up.

4.10. Quality control

Data collectors were trained by the principal investigator before the actual work. Standard operating procedures (SOPS) were strictly followed. Quality was assured at each stage (pre-analytical, analytical and post analytical stages) of diagnosis of the patient sample. Principal investigator and experienced laboratory technologists working in Hossana Health Centre examined each blood smear. All *P. vivax* positive slides on day of admission and few negative slides were picked randomly from slides prepared during follow-up and all slides on day of recurrence were re-examined by Principal investigator and senior laboratory technologists in the Health centre.

4.11. Data analysis

Statistical Package for Social Science (SPSS) software version16 was used for data management and analysis. Data of patient having mixed infection with *P. falciparum*, lost to follow-up and vomiting was excluded from the analysis. The analysis included the proportion of early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR) at day 28. Kaplan-Meier survival estimate was used to evaluate risk of therapeutic failure in study participants during follow-up period. Change in mean haemoglobin level in days 0 and 28 was compared using paired t- test. In non-normally distributed data (age), median was used to measure the central tendency. Parasite counts of study participants were made using geometric mean. In all analysis, p-values < 0.05 was considered significant.

4.12. Operational definition

Recurrence- New parasitaemia of unknown origin

4.13. Study end-points

A study end-point is the classification assigned to a patient. Valid study endpoints include treatment failure (early treatment failure, late clinical failure, late parasitological failure), completion of follow-up without treatment failure (adequate clinical and parasitological response), loss to follow-up, withdrawal from the study and protocol violation (WHO, 2009).

4.13.1 Early Treatment Failure (ETF)

- Signs of severe malaria on day 1, 2 or 3, in the presence of parasitemia
- Parasite density at day 2 higher than day 0
- Parasitemia on day 3 with axillary temperature ≥ 37.5°C;
- Parasite density at day 3 ≥ 25% of count on day 0.

4.13.2 Late Clinical Failure (LCF)

- Signs of severe malaria on any day between 4 and 28
- Presence of parasitaemia between day 4 and 28, with axillary T^o >37.5°C

4.13.3 Late Parasitological Failure (LPF)

- Presence of parasitaemia on any day between day 7 and 28 and axillary T^o < 37.5°C, without previously meeting any of the criteria of ETF or LCF.

4.13.3 Adequate Clinical and Parasitological Response (ACPR)

- Absence of parasitaemia on day 28, irrespective of axillary temperature, without previously meeting any of the criteria of ETF, LCF or LPF.

4.14. Dissemination of Result

Final finding of the study will be disseminated through presentation at Jimma University during defence, reporting to Hadiya Zone Health Office, Hossana Town Administration Health Office, Hossana Health Centre and others via scientific presentations and publications.

4.15. Ethical clearance

The study was reviewed and approved by the Ethical Review Committee of Jimma University, College of Public Health and Medical Sciences. Permission was obtained from Hadiya Zone Health Office, Hossana Town Administration Health Office, and Hossana Health Centre. The objectives of the study was explained to the participants and written informed consent obtained from each patient and parents or guardians, in cases when the study subjects are children (Annex I and II).

CHAPTER FIVE

RESULT

A total of 1693 patients screened for Malaria at Hossana Health Centre, 1412 were negative and 281 were positive for malaria. Among all positive patients 182 were positive for *P.falciparum*, 92 *P.vivax* and 7 are mixed infection. From 92 *P.vivax* positive patients, 63 patients who fulfilled inclusion criteria were selected. 29 patients were excluded from the study before enrolment as they did not fulfil the inclusion criteria. Out of this, 13 were excluded due to long distance from the health centre, 7 because of pregnancy; 6 because of prior antimalarial intake and 3 because of refusal of consent**.** From 63 study participants, 3 patients were excluded from the study because, 1 participant vomited twice during the third dose of chloroquine at day 2, the second was infected by *P.falciparum* asexual stage on day 14, and the third patient was lost to follow up on day 14, 60 participants completed the 28 day follow up study (Figure 3).

Among the recruited study participants, males were higher in proportion compared to females (35 were males and 25 were females). The median age of study participants was 23 (range: 4 to 59) (Table 1). Among the study participants, 32 (53.3%) had a history of fever and 28 (46.7%) had fever at the time of enrolment. The duration of illness of the patients before enrolment was 3.05 ± 1.41 (mean ± SD) days. The geometric mean of parasite at day 0 was 3472.1parasites/µl.

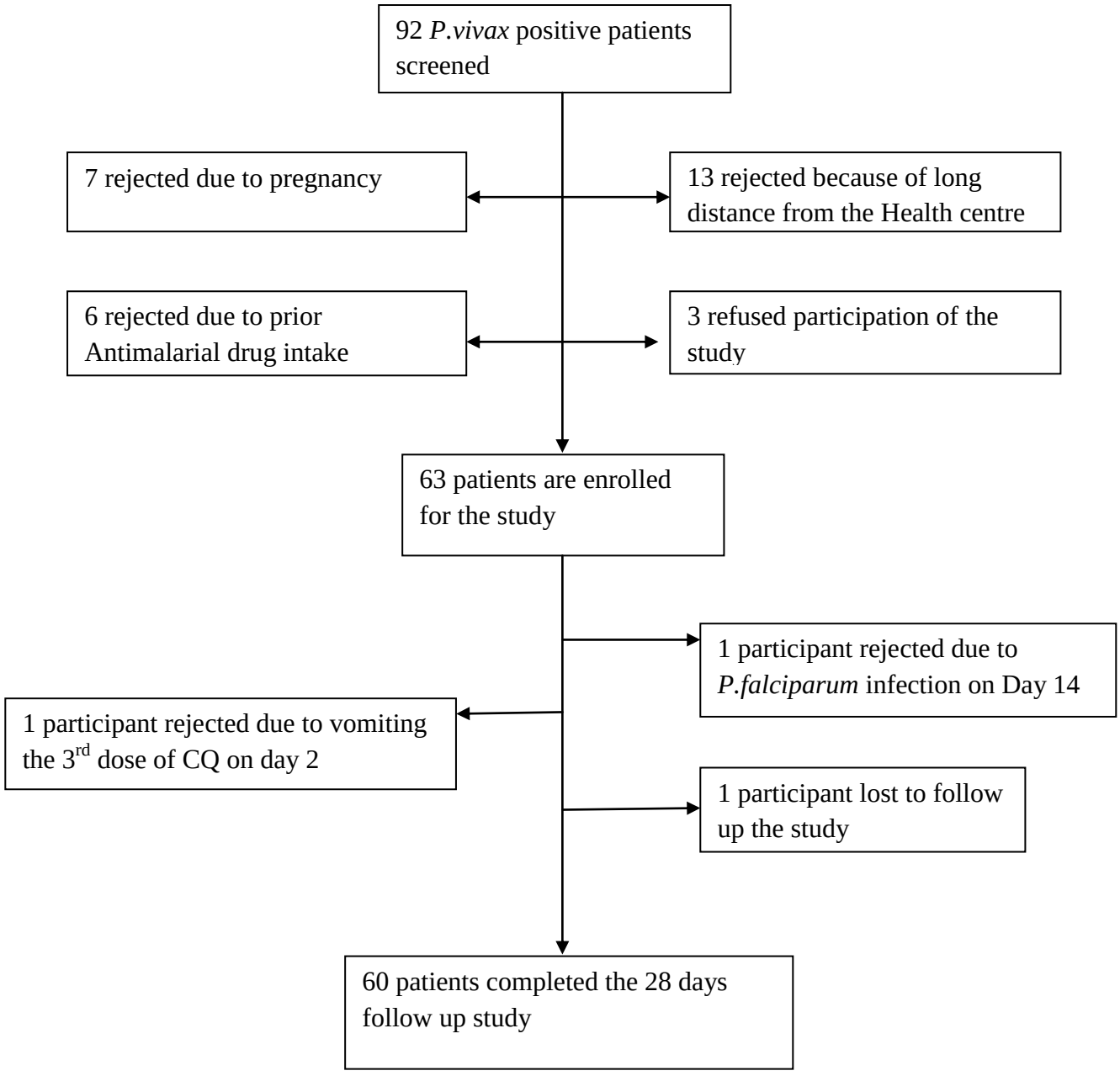

Figure 2. Flow chart shows patient enrolment for therapeutic efficacy study of chloroquine in Hossana Health Centre Southern Ethiopia from April to June 2014.

Table 1. Socio demographic characteristics of patients enrolled in the invivo therapeutic efficacy test of chloroquine in *P. vivax* malaria in Hossana Health Centre Southern Ethiopia from April-June 2014.

Sociodemographic variables	Total No (%)
Age	
Median	23
Range	4-59
Gender	
Male	35 (58.3)
Female	25 (41.7)
Ethnicity	
Hadiya	40 (66.7)
Kembata	11 (18.3)
Gurage	7 (11.7)
Silte	2 (3.3)
Occupation	
Government employee	2 (3.3)
Unemployed	3 (5.0)
Student	27 (45.0)
House wife	15 (25.0)
Farmer	8 (13.8)
Daily Labourer	4 (6.7)
Marital status	
Single	28 (46.7)
Married	31 (51.7)
Widowed	1

Based on therapeutic failure risk calculated by the Kaplan Meier survival analysis, the number of patients at risk on day 0 was 63 and these fell to 59 at day 28. Among the 60 patients, 100% parasite clearance was observed by day 3. However, 2 (3.3 %) of chloroquine treatment failure was observed during the follow up period. This was observed in 10 and 14 years children in day 28 and 21 respectively. This places the risk of chloroquine failure on day 28 at 3.3% (Table 2).

Table 2. Kaplan-Meier survival estimate of risk of therapeutic failure of chloroquine for treatment of *P. vivax*, at Hossana Health Centre Southern Ethiopia April to June 2014.

D	N	TF	Ex	IR_D	FCI_D
Day 0	63	0	0	1.000	0
Day 1	63	0	0	1.000	0
Day 2	63	0	1	1.000	0
Day 3	62	0	0	1.000	0
Day 7	62	0	0	1.000	0
Day 14	62	0	2	1.000	0
Day 21	60	1	0	0.983	0.017
Day 28	59	1	0	0.967	0.033
Total		**2**	**3**		

Keys D= Day of test, N = Number of subjects remaining at risk, TF = Incident cases of therapeutic failure, Ex = Excluded due to vomiting, loss to follow-up, and infection with *P.falciparum*, IRD = Interval risk (IR at Day 0 = 1), $IR_D = [(N_D – Ex) – TF_D] / (N_D – Ex)$, FCI_D = Cumulative incidence of therapeutic failure ($1-IR_D$)

5.1. Treatment Outcome

On the first day of the study period, relatively higher parasitaemia (10720 Parasite /μl) and lower mean haemoglobin concentration (Mean 11.5 mg/dl) were observed in the study participants. Moreover, following chloroquine treatment, parasitaemia and fever of study participants were cleared and the mean haemoglobin concentration of them was improved (13.4 mg/dl). In this study, there were no early treatment failure and late clinical failures but, 2 (3.3%) late parasitological failures were observed at the end of the study. The adequate clinical and parasitological response after 28 days follow up was 58 (96.7%).

Table 3. Proportion of chloroquine treated *P.vivax* infected study participants in 28 days study Hossana Health Centre, Southern Ethiopia from April to June 2014

Treatment status	**Age in**			**Total**
	>5=1(1.6%)	**5-14=16(26.7%)**	**≥15=43(71.7%)**	**60(100%)**
ETF	0(0%)	0(0%)	0(0%)	0(0%)
LCF	0(0%)	0(0%)	0(0%)	0(0%)
LPF	0(0%)	2(3.3%)	0(0%)	2(3.3%)
ACPR	1(1.7%)	14(23.3%)	43(71.7%)	58(96.7%)
Total	1	16	43	60

ETF-Early Treatment Failure, **LPF**-Late Parasitological Failure, **LPF**-Late Parasitological Failure, **ACPR**-Adequate Clinical and Parasitological Response

5.2. Parasite Clearance

Parasitaemia clearance time was 72 hours for almost all patients involved in the 28 days follow up in-vivo study. At day 0, before drug administration, the geometric mean of parasitaemia of the study participants was 3708 parasites/µl of blood , (Maximum 10720 parasites/µl, Minimum 1600 parasites/µl). The geometric mean of parasitaemia on day 2 dropped to 1277 parasites/ µl. On the third day of the study period, 100 % of parasitaemia was cleared from the study participants, as shown in Figure 3.

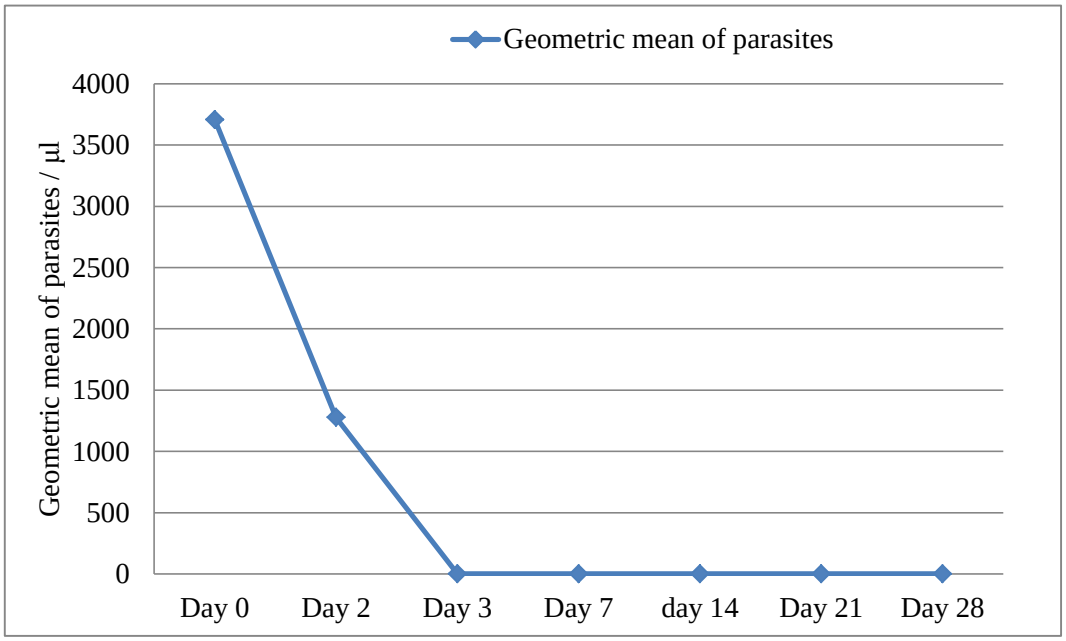

Figure 3: Parasite clearance following Chloroquine treatment of *P.vivax* malaria infected patients in Hossana Health Centre Southern Ethiopia from April to June 2014

5.3. Fever Clearance

Mean body temperature of the study participants at the time of enrolment was 37.7°C (minimum 36.5°C, maximum 39.1°C). Among the study participants, 32(53.3%) had a history of fever and 28(46.7%) had fever at the day of enrolment. Almost all study participants cleared fever (Mean body temperature in day 2 was 37.0°C) following parasitaemia clearance and 36 °C mean body temperature were recorded as it is shown in Figure 4 .

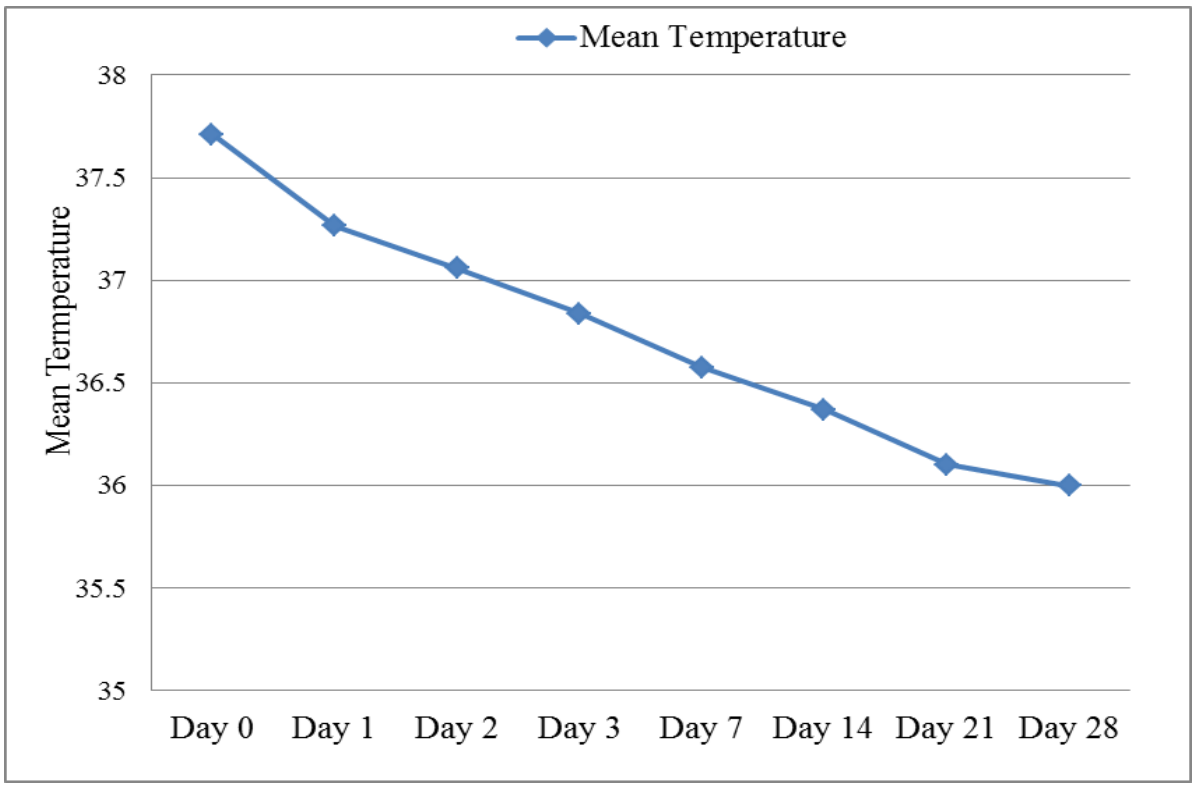

Figure 4: Fever clearance following chloroquine treatment of *P. vivax* malaria infected patients in Hossana Health Centre Southern Ethiopia from April to June 2014

5.4. Haemoglobin recovery

On day of enrolment , 30 (50%) patients were found to have mild anaemia; 1 patient was found to have moderate anaemia and no patients had severe anaemia and 29 (48.3%) of them are non-anaemic (Hgb value ≥12 g/dl). On the other hand significant (P = 0.01) increase was observed in the haemoglobin level between the baseline and day 28. Among the study participants with adequate clinical and parasitological response 52 (86.7%) of them were non anaemic and 8 (13.3%) had mild anemia.

A slight decrease in haemoglobin value from baseline to day of recurrence (Day 21) was detected in one patient (from 10.6 to 9.7 g/dl) and the level finally increased to 10.2 g/dl in day 28. Another patient with recurrence of malaria was detected with slight increase in haemoglobin concentration (from 9.9 to 10g/dl). The mean haemoglobin concentration of our study participants at day of enrolment was 11.5 g/dl (ranging from 9.9 g/dl to 13.2 g/dl) and 13.4g/dl (ranging from 10g/dl to 14g/dl) on day 28. These observed improvements in haemoglobin concentration are consistent with parasite clearance.

Table 4: Recovery of haemoglobin concentration observed in *P.vivax* infected study participants following chloroquine treatment in Hossana Health Centre, Southern Ethiopia from April to June 2014

Haemoglobin category	Day of enrolment (Day 0)			Total	End of follow up (day 28)			Total
	1-4	5-14	>15		1-4	5-14	>15	
Mild	0	9(30%)	21(70%)	30(50%)	0	4(50%)	4(50%)	8(13.3%)
Moderate	0	1(1.6)	0	1	0	0	0	0
Sever	0	0	0	0	0	0	0	0
Non anemia	1(3.4%)	6(20.7%)	22(75.9%)	29(48.3%)	1(1.9%)	12(23.1%)	39(75%)	52(86.7%)

CHAPTER SIX

DISCUSSION

Chloroquine has been in use both for treatment and prophylaxis in health institutions in Ethiopia. It is antimalarial drug recommended as first line treatment by the Ministry of Health of Ethiopia for treatment of *P. Vivax* malaria infection in the country (DACA, 2010a). However, some reports on chloroquine resistant *P. vivax* malaria are reported from different malaria endemic areas of Ethiopia (Tulu *et al*., 1996, Teka *et al*., 2008, Ketema *et al*., 2009, Ketema *et al*., 2011). It is becoming a major public health problem which requires rapid and effective management to control the spread. This requires proper diagnosis of cases and administration of effective antimalarial drugs (WHO, 2009).

The present study showed prevalence of *P. falciparum* and *P.vivax* malaria in Hossana Health Centre, Southern Ethiopia. *P. falciparum* infected patients were detected more frequently than *P.vivax* patients during enrolment of our study participants. Out of 282 malaria positive patients, 182 of them were *P. falciparum* patients and 92 patients had *P. vivax* infections and the rest 7 had mixed infections. According to Hossana Town health office report, *P. falciparum* is more prevalent than *P.vivax* in previous report which supports the recent study.

A 28 days invivo therapeutic efficacy test on chloroquine undertaken in Hossana Health Centre has demonstrated 2 (3.3%) late parasitological failures. The low level of treatment failure detected in the present study was comparable with previous reports from different parts of Ethiopia. Debrezeit 2% (Tulu *et al*., 1996), Serbo 3.6% (Ketema *et al*., 2009), Debrezeit 4.6 % (Teka *et al*., 2008). And also comparable with studies conducted in Madagascar 5.1% (Barnadas *et al*., 2008) and Maynamar 0.8% (Guthmann *et al*., 2008). On the other hand, our result is low when it is compared with a high treatment failures reported from Halaba special woreda in South Ethiopia 11.7% (Ketema *et al*., 2011), in Turkey Sanliurfa province 9.5% (Dilmaec *et al*., 2010) ,Colombia 11% (Soto *et al*., 2001) ,Brazil 10.1% (Simoes *et al*., 2007), Guyana 2 out of 3 (Elizabeth *et al*., 1996),Indonesia 23%(David *et al*., 1998) ,Eastern Indonesia 56% (Sutanto *et al*., 2009) and Indonesian Papua 84% (Sumawinata *et al*., 2003) .

In our study, 2 patients were observed with treatment failure were children with 10 and 14 age respectively which is similar with treatment failures observed in studies conducted in Debrezeit and Serbo respectively (Teka *et al*., 2008, Ketema *et al*., 2009), India (Shah, 2008) and Indonesia (David *et al*., 1998).

Decreased parasites density on day of recurrence was observed in our study in one patient unlike studies done by (Ketema et al., 2009, Soto et al., 2001, Baird, 2009). Based on WHO guideline for therapeutic efficacy study of antimalarials; resistance is classified as early treatment failure, late clinical failure, late parasitological failure or adequate clinical and parasitological response. Our study reveals two patients with late parasitological failure which is defined as presence of parasitaemia on any day from day 7 to day 28 and axillary temperature < 37.5°C, without previously meeting any of the criteria of early treatment failure or late clinical failure (WHO, 2009, WHO, 2010α).

Anaemia is a major effect of malaria infection. During intra-erythrocytic development, malaria trophozoites digest haemoglobin. Thus treatment with the appropriate drug is expected to improve the patients' haemoglobin level with time (Moore *et al*., 2006). In this study significant increase (p=0.01) was observed in the haemoglobin level from baseline to day 28 among patients with adequate clinical and parasitological response. Also in our study one patient with treatment failure showed improvement of haemoglobin value on day of recurrence. However, one patient with treatment failure had no haemoglobin improvement on day of recurrence.

Malaria infection and severity decline with increasing age among populations who live in endemic areas because of acquired immunity to malaria due to repeated exposure (Mannan *et al.*, 2003). But, there is risk of malaria infection in children and pregnant mothers because of low immune response. Also treatment of malaria with antimalarials gives better parasite clearance in immune individuals. But, those who are non-immune suffered from malaria infection as well as recurrence (Marsh and Kinyanju, 2006). This may be the reason for LPF observed in our study in children age 10 and 14 respectively in which acquired immunity to malaria is low.

Fever and malaria parasite clearance are associated because, malaria toxins and metabolic end products are related with increasing fever and disappear following parasite clearance because some of them have short half-life (Kwiatkowski *et al*.,

1997). Also low grade fever may appear in patients after clearance of parasitemia due to circulating toxins and molecules of malaria parasites that have long half-life (Olaleye *et al*., 1998). The fast rate of parasite and fever clearance following chloroquine treatment detected in present study is comparable with earlier studies from Ethiopia (Teka *et al*., 2008, Ketema et al., 2009) and other countries (David *et al*., 1998, Guthmann *et al*., 2008, Barnadas *et al*., 2008).

Complaints of malaria symptoms such as headache, fever, chilling, and joint pain, during the follow-up period without confirmed parasitaemia in their blood was noticed in study conducted in Halaba (Ketema *et al*., 2011). But in our study none of patients with treatment failure complained of malaria signs and symptoms during follow up which is similar with study conducted in Serbo (Ketema *et al*., 2009). In all patients with treatment failure, parasite count increased from day of admission to day of parasite recurrence in study conducted in Serbo. Similarly one of our patients has showed increased parasite load but the rest one had no increased parasitaemia.

Our study showed chloroquine treatment failures of *P. vivax* malaria in Hossana Health Centre Southern Ethiopia. However the level of resistance is not high compared with some other previous studies in Ethiopia, 13.7% in Halaba (Ketema *et al*., 2011). It is important to caution the responsible authorities to monitor the magnitude of drug resistance problem in all malaria endemic parts of the country. According to WHO, first-line treatment of malaria should be changed if the total failure rate exceeds 10% (WHO, 2009). So, it is important to consider the problem as public health importance to limit the magnitude.

Limitations of the study

The following laboratory analyses were not performed because of financial constraints.

- Genotyping of treatment failure cases were not performed to distinguish relapse and re infection from resistance.
- Drug concentration is not performed in failure cases to know the whole blood drug concentration level.

CHAPTER SEVEN

CONCLUSIONS & RECOMMENDATIONS

7.1. Conclusions

Based on the findings of the present study, the following conclusions reached

- The three dose regimen of chloroquine showed therapeutic efficacy (96.7%) in the treatment of uncomplicated *P.vivax* malaria in Hossana Health Centre, Southern Ethiopia.
- A 3.3% chloroquine treatment failure detected in Hossana Health Centre Southern Ethiopia is late parasitological failure.
- Rapid clearance of fever and asexual parasitaemia was observed after chloroquine treatment of uncomplicated *P.vivax* malaria.
- Improvement in mean haemoglobin level was achieved following chloroquine treatment from Day 0 to day 28.

7.2. Recommendations

- Regular monitoring of the pattern of resistance to chloroquine is needed in *P.vivax* malaria endemic areas of the country to take measures rapidly and effectively to control the spread.
- Proper instruction and utilization of chloroquine should be exercised in order to avoid resistance.
- The prevalence of *P. falciparum* and *P.vivax* malaria observed in the study area requires strong malaria intervention measures.

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ANNEXES

Annex I: Respondents Information sheet (English Version)

Title of the project: Therapeutic efficacy of chloroquine for treatment of *P. vivax* malaria in Hossana Health Centre, Southern Ethiopia

Name of Principal Investigator: Mesfin Assefa

Organization: Jimma University College of Public Health and Medical Sciences Department of Medical Laboratory Sciences and Pathology and Hossana Town Administration Health office.

Name of sponsor: Jimma University

This information sheet is prepared for *P.vivax* positive patients who involved in project entitled above. We are going to tell you about the whole process that will happen in the study and requesting you to participate voluntarily.

Description and Purpose of the study

Chloroquine is the first line for treatment of *P. vivax* malaria in Ethiopia. Few reports are indicating the emergence and spread of chloroquine resistant *P. vivax* strains in malaria endemic areas of Ethiopia. Therefore, this study is needed to assess the degree of distribution of the resistance pattern of *P.vivax* in endemic areas of the country.

Procedures

If you are willing to participate in the study, you will be asked to sign a consent form and the following procedures will be done.

- Your medical history will be taken
- You will provide us 5 minutes interview
- We will take 2-3 drops of blood sample in each follow up days
- The collected sample will be processed in Hossana health centre
- Haematological and parasitological tests will be done from collected blood sample.

- The collected blood will be used only for identification of *P. vivax* and measure haemoglobin level, never be used for other purpose

Risks and discomforts

There is no risk and discomfort in participating in this study. During all sample collection we will follow Standard operational procedures.

Benefits and Compensation

By participating in this study, there will not be direct financial benefit. If you are under treatment failure you will be treated by second line drug.

Confidentiality

All information collected from the study subjects will be kept confidential. Any information about the participant that will be collected from the study will be stored in a file that will not bear a name on it, but only a number assigned to it instead.

Voluntary participation and withdrawal:

Your participation in this study is voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put at risk at any present or future medical care and other benefits in the Health centre. You should ask the study investigators listed below any questions you may have about this research study. You may ask questions in the future if you do not understand something that is being done.

Use the following address for any question.

Mr Mesfin Assefa, Phone No +251 911 09 96 86, Email: mesfinassefa15@gmail.com

Mr Abdissa Biruksew, Phone No +251 911 964 174, Email: abdissa.hordofa@ju.edu.et

DR Teferi Eshetu, Phone No +251 923 998 057, Email: teferi.eshetu@ju.edu.et

For the success of our study, we will be asking you to give the correct answer for the respective questions. Thank you for your assistance.

የጥናቱ ተሳታፊዎች መረጃ ቅፅ (የአማርኛ ግልባጭ)

የጥናቱ ርዕስ :የወባ በሽታ መድሐኒት(ክሎሮኪዩን) ፈዋሽነት ጥናት በሆሳዕና አከባቢ

የዋና ተመራማሪ ስም: መስፍን አሰፋ

የድርጅቱ ስም፡- የጅማ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ሳይንስ እና ፓቶሎጅ ትምህርት ክፍል እና የሆሳዕና ከተማ አስተዳደር ጤና ጥበቃ ቢሮ

ይህ የመረጃ ቅፅ የተዘጋጀው ከላይ በተጠቀሰው ጥናት ለሚሳተፉ ተሳታፊዎች ፋይዳ ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሂዳቸው ስለፈለግናቸው ጉዳዮች እና ስለ ጥናቱ ጠቅላላ ማብራርያ ይሰጣል:: በመሆኑም በጥናቱ የሚሳተፉት በራስዎ ፍላጐት ብቻ መሆኑን በትህትና እንገልፃለን፡፡

ስለ ጥናቱ በጥቂቱ

ክሎሮኪዩን የተባለው መድሃኒት ቫይቫክስ ለሚባለው የወባ አይነት በኢትዮጵያ ውስጥ ጥቅም ላይ ያለ መድሃኒት ነው።ይሁን እንጂ ይህንን መድሃኒት የተላመደ ወባ በአንዳንድ የሀገሪቱ ክፍል እየተከሰተና እየተሰፋፋ ይገኛል። ስለሆነም ይህ ጥናት በዚህ አከባቢ ያለውን ክሎሮኪዩን የተላመደ ወባ ስርጭትና መጠን ያጠናል።

የጥናቱ ሂደት ዝርዝር

በጥናቱ ለመሳተፍ ከተስማሙ የሚከተሉትን መረጀዎችና ናሙና እንወስዳለን፡

- የህክምና ታሪኮ ይመዘገባል
- እንዲሁም ከራስዎ አንደበት የ5 ደቂቃ ቃለ መጠየቅ ይደረግሎታል
- ከ 2-3 ጠብታ የደም ናሙና በ ቀጠሮ ቀናት ይሰጣሉ
- የ ሄማቶሎጂና ፓራሳይቶሎጂ ምርመራ ይደረግሎታል
- የተሰበሰበው የደም ናሙና በ በሆሳዕና ጤና አጠባበቅ ጣቢያ ላቦራቶሪ ምርመራ ይካሄዳል
- የተሰበሰበው የደም ናሙና ለ ወባ ምርመራ እና ለ ሄሞግሎቢን መጠን ልኬት ይሆናል እንጂ ከተሰበሰበበት ኣላማ ውጪ አይውልም

ስጋትና ጉዳት

በአጠቃላይ ከላይ የተጠቀሰዉን ምርመራ ለማድለግ የደም ናሙና በሚወሰድበት ጊዚ ሊያጋጥም የሚችል የነላ ችግር ባይኖርም ትንሽ የህመም ስሜት ይኖረዋል፡፡

ሊያስገኛቸው የሚችሉ ጥቅሞች እና የካሳ ክፍያ

በዚህ ጥናት ውስጥ በመሳተፍዎ በጥሬ ገንዘብ የሚደረግ የካሳ ክፍያ አይኖርም ::

የጥናቱ ምስጢራዊነት

ማንኛውም በጥናቱ የሚገኙ መረጃዎች በምስጢር ይጠበቃሉ:: የጥናቱ መረጃዎች በሙሉ የሚቀመጡት ከእርሶ ስም ጋር ሳይሆን ለጥናቱ ተብሎ በሚሰጠው ስውር ቁጥር ሲሆን ጥናቱን ከሚያስከሄዱት ባለሙያዎች በስተቀር ማንም ሊያውቅ አይችልም፡: የእርስዎን ማንነት በሚገልጥ መልኩ የተዘጋጀውን መረጃ በፊርማዎ የተረጋገጠ ፍቃድ ሳናገኝ ይፋ አናደርግም:፡ ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢወጣ ወይንም በሚድያ ቢነገር የእርስዎ ስም በምንም መልኩ አይጠቀስም፡:

ያለመቀበል ወይም ጥሎ የመውጣት መብት

በዚህ ጥናት ውስጥ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ይሆናል:፡በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብትዎ ሙሉ በሙሉ የተጠበቀ ነው:: በጥናቱ ባለመሳተፍዎ ወይም ከጥናት በመገለልዎ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታ ላይ ተፅዕኖ አይኖረውም ከዚህ በፊት ሲያገኙ ከነበሩት ጥቅሞች አንዳች ነገር አይጐሎቦትም ጥናቱን የሚያከናውነው አካል ወይም ድጋፍ ሰጭ አካል ከእራስዎ ጥቅም ሲባል በጥናቱ እንዳይሳተፉ ሊከለከል ይችላል፡፡

ጥያቄ ካለዎት

ስለጥናቱ ማንኛውንም ጥያቄ ወይም እርስዎ በዚህ ጥናት ውስጥ ለሚኖርዎት ድርሻ አሳሳቢ ጉዳት ወይም ቅሬታ ካለዋት የሚከተሉትን ስልኮች ወይም ኢሜል አድራሻ በመጠቀም የጥናቱን ባለቤቶች ማነጋገር ይችላሉ፡፡

1. አቶ መስፍን አሰፋ፤-ስልክ+251 911 09 96 86-ኢሜል: mesfinassefa15@gmail.com
2. አቶ አብዲሳ ብሩክሰው፤-ስልክ +251 911 964 174-ኢሜል: abdissahordofa@ju.edu.et
3. ዶ/ር ተፈሪ እሸቱ፤-ስልክ +251 923 998 057 ኢሜል: teferi.eshetu@ju.edu.et

Annex II: Patient consent form (English Version)

Participant Code Number_____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with **Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria among patients at Hossana Health Centre, Southern Ethiopia.** I have been informed that medical history and blood samples will be taken and there will be minimal pain during sample collection. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

Therefore I give my consent freely for my participation in this study.

Patient Name _____ signature _____ Date_____

Investigator name _____signature _____Date_____

የስምምነት ቅፅ (የአማርኛ ግልባጭ)

የተሳታፊው ልዩ መለያ ቁጥር _____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የወባ በሽታ መድሐኒት(ክሎሮኪዩን) ፈዋሽነት ጥናት በሆሳዕና አከባቢ በሚል ርዕስ በታሰበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ አግኝቻለሁ፡፡ የህክምና መረጃና የደም ናሙና ምንም አይነት ጉዳት በማያደርስ መልኩ እንደሚወሰድ ተረድቻለሁ፡፡ በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጥር እንደሚያዙ ተነግሮኛል፡፡ እንድሁም የምጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ ከጥናቱ በማናቸውም ወቅት ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ ወደ ፊትም ሆነ አሁን የማገኛቸው የህክምና ግልጋሎቶች እንደማይጓደሉብኝ ጭምር ተነግሮኛል፡፡

በመሆኑም በዚህ ምርምር ለመሳተፍ ወስኛለሁ፡፡

የታካሚ/ የተሳታፊ ስም _____ፊርማ _____ቀን_____

የተመራማሪ ስም _____ፊርማ _____ቀን _____

Annex III: Questionnaire (English Version)

JIMMA UNIVERSITY COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY

Data collection tool on therapeutic efficacy of chloroquine for treatment of *P. vivax* malaria in Hossana Health Centre Southern Ethiopia.

General instructions- For all the close ended questions please give your responses by choosing the letter of your answer (s) and for open ended questions use the space provided.

Part one: General Information

I. Address

a. Kebele_____
b. House number_____
c. Telephone number_____

II. Sociodemographic information

1. Age:_____
2. Sex: M_____F_____
3. Ethnic group:
    a. Hadiya
    b. Kembata
    c. Gurage
    d. Silte
    e. Other specify_____
4. Religion
    a. Muslim
    b. Orthodox
    c. Protestant
    d. Other specify_____

5. Occupation

a. Government employee

b. Unemployed

c. Student

d. House Wife

e. Farmer

f. Daily labourer

g. Other specify_____

6. Marital status

a. Single

b. Married

c. Divorced

d. Widowed

5. Education status

a. Higher Education

b. High school

c. Elementary

d. Illiterate

መጠይቅ (የአማርኛ ግልባጭ)

በጂማ ዩኒቨርሲቲ በሕብረተሰብ ጤናና ሕክምና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስና ፓቶሎጂ ትምህርት ክፍል

የወባ በሽታ መድሐኒት(ክሎሮኪዩን) ፈዋሽነትን በሆሳዕና አከባቢ ለማጥናት የተዘጋጀ መጠይቅ፡፡

<u>አጠቃላይ መመርያ</u>

በመጠይቁ ላይ ላሉት የምርጫ ጥያቄዎች የህሙማንን መልስ የያዘውን ፊደል በመምረጥ ይመልሱ፡፡የጽሑፍ ጥያቄዎችን ደግሞ ለመልስ በተሰጠው ቦታ ላይ ይጻፉ፡፡ መረጃ ሰብሳቢዎች መጻፍና ማንበብ ለማይችሉ ታሳታፊዎች ጥያቄዎችን በማንበብና መልሶቻቸውን በመጻፍ ይተባበራሉ፡፡

ክፍል አንድ – ጠቅላላ መረጃ

ክፍል ፩. <u>አድራሻ</u>

የመጡበት ቀበሌ ስም _____

ቤት ቁጥር_____

የስልክ ቁጥር_____

ክፍል2. <u>ማሕበራዊ ጉዳዮች</u>

1.ዕድሜ _____

2.ብሔር

ሀ. ሀድያ ለ. ከምባታ ሐ. ጉራጌ መ. ስልጤ ሠ. ሌላ ከሆነ _____

3.ሃይማኖት

ሀ.እስልምና ለ.አርቶዶስ ሐ.ፕሮቴስታንት መ.ሌላ ከሆነ _____

4.ሥራ

ሀ. የመንግስት ሰራተኛ

ለ.ተቀጣሪ ያልሆነ

ሐ.ተማሪ

መ.የቤት እመቤት

ሠ.አርሶ አደር

ረ.የቀን ሠራተኛ

ሰ.ሌላ ከሆነ _____

6.የትዳር ሁኔታ

ሀ.ያገባ/ች

ለ.ያላገባ/ች

ሐ.የፈታ/ች

መ.ባል የሞተባት

7.የትምህርት ደረጃ

ሀ. ዩኒቨርሲቲ

ለ.2ኛ ደረጃ

ሐ . 1ኛ ደረጃ

መ. ያልተማረ/ች

Annex IV: Blood collection & smear preparation

1. Recording the patient's details on the form or register, label pre-cleaned slides with patient's name , date and time of collection
2. By wearing protective latex gloves, hold the patient's left hand, palm facing upwards, and select the third finger from the thumb, called the 'ring finger'. For infants, the big toe can be used.
3. Clean the fingers with cotton wool socked with alcohol and dry the finger with clean gauze.
4. Using a sterile lancet puncture the ball of the finger or toe.
5. Apply gentle pressure to the finger or toe and express the first drop of blood; wipe it away with dry cotton wool, making sure that no cotton strands remain that might later be mixed with the blood.
6. Collect a single small drop of blood on the middle of the slide and make thin film. Apply further gentle pressure to express more blood, and collect a drop of blood on the slide, about 1 cm away for thick film. Wipe the remaining blood off the finger with cotton wool.
7. Prepare a thin smear with spreader slide by placing it in front of the small drop and back to the blood until it spread at the lower edge of the spreader slide and smear the blood along with the length of the slide by holding the spreader at 45^0. For thick smear, spread the blood with the corner of another microscope slide to form a rough circle. The size of the smear should be 1-2 cm diameter. Do not make the smear too thick.
8. Keep the slide on drying rack at horizontal position to be dried with air but it should be free of dust and protected from flies.
9. Fix only the thin smear with concentrated methyl alcohol for 30 seconds.

Annex V: Procedure for Giemsa staining

1. Prepare a 10 % solution of Giemsa stain by adding 9 ml of Giemsa stock solution to 91 ml of water buffered to pH 7.2, or multiples of this.
2. Place the slides back to back in a staining trough, making sure that the thick films are together at one end of the trough.
3. Pour the stain into the trough. Do not pour it directly onto the thick films, as they may float off the slides.
4. Stain the slides for 10 minutes
5. Remove the slides, drain the reagent and rinse gently with tap water that kept in a dish.
6. Drain the water and place the slides on drying rack at vertical position.
7. After being dried, examine each slide microscopically using a 100x oil immersion lens and 10x eyepieces to screen parasitaemia, to identify the parasite species and to determine density of the parasite.

Annex VI: Hemoglobin Measurement by HemoCue Analyzer

Test procedure:

1. Seat the patient comfortably; use the middle finger or the ring finger for sampling.
2. The patient fingers should be straight but not stressed to avoid stasis.
3. Remove a micro cuvette from the vial and recap immediately.
4. Clean site with alcohol soaked gauze for blood collection.
5. Using your thumb, lightly press the finger from the top to stimulate flow of blood to the sampling point.
6. Puncture the fingertip by using sterile blood lancet and discard the lancet in sharp container.
7. Wipe away the first two large drops of blood. This reduces the possibility of a dilution effect by interstitial fluid. If necessary, apply light pressure again, until another drop of blood appears.
6. Make sure the third drop of blood is big enough to fill the micro cuvette completely. Hold the micro cuvette at the "wing" end and touch the tip into the middle of the drop of blood from above the finger. Keep the micro cuvette in contact with the blood and fill in one continuous process. Do not refill a partially filled micro cuvette.
7. Wipe any residual blood from the sides of the micro cuvette with a piece of gauze. Don't touch the tip of the cuvette with the gauze because it withdraws blood.
8. Visually inspect for air bubbles in the centre of the cuvette eye. If bubbles are present in the cuvette eye, discard the micro cuvette and obtain another specimen.
9. The filled micro cuvette should be analysed within three minutes. Place the filled micro cuvette into the cuvette holder and gently slide the holder into the measuring position.
10. The Hemoglobin value displayed in grams/dl after approximately 30- 50 seconds.
13. Record the result before removing the micro cuvette from the instrument.
14. Dispose of the micro cuvette in the biohazardous waste container.
15. If an "ERROR" code is displayed, refer to the manufacturer's manual

Annex VII: WHO haemoglobin threshold used to define anemia in different age and gender groups (WHO, 2011b)

Age or gender group	Non anaemia (g/dl)	Anaemia (g/dl)		
		Mild	Moderate	Sever
Children (0.5–5.0 years)	>11.0	10-10.9	7-9.9	<7
Children (5.0–12.0 years)	>11.5	11-11.4	8-10.9	<8
Children (12.0–15.0 years)	>12.0	11-11.9	8-10.9	<8
Women, non-pregnant (>15years)	>12.0	11-11.9	8-10.9	<8
Women, pregnant (>15)years	>11.0	10-10.9	7-9.9	<7
Men (>15years)	>13.0	11-12.9	8-10.9	<8

Annex VIII: Laboratory request form

Code of the patient_____

Age_____Sex _____ Address/ kebele_____

Tele .no_____ Contact person_____

Type of test_____

Date & time of sample collection_____

Result_____

Name & signature of investigator_____

Annex IX: Declaration sheet

I the undersigned declare that this thesis is my own work and all sources of materials used for the thesis have been fully acknowledged. I agreed to accept responsibility for the scientific and ethical conduct of the research.

Name of student: Mesfin Assefa

Signature_____

Date _____

This thesis has been submitted with my approval as University advisor

First advisor

Name: Mr. Abdissa Biruksew (BSc, MSc)

Signature _____

Date_____

Second advisor

Name: Dr. Teferi Eshetu (PhD)

Signature _____

Date_____

Internal Examiner

Name: Mr. Ahemed Zeyinudine (BSc, MSc, Associate Professor of Medical Parasitology)

Signature _____

Date _____