JIMMA UNIVERSITY

COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY

THERAPEUTIC EFFICACY OF CHLOROQUINE FOR TREATMENT OF *PLASMODIUM VIVAX* MALARIA IN SHOA ROBIT, NORTH EASTERNE THIOPIA

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ABSTRACT

Background: *About 40% of all malaria infection in Ethiopia is caused by Plasmodium vivax. Chloroquine (CQ) is the first line treatment for confirmed P. vivax malaria in the country. However, chloroquine resistant P. vivax (CRPv) has started to affect the efficacy of this drug. The present study was carried out to assess the therapeutic efficacy of chloroquine for treatment of Plasmodium vivax malaria in Shoa Robit, North-East Ethiopia.*

Methods: *An in vivo drug efficacy study was conducted in Shoa Robit from October 2013 to February 2014. Eighty-seven patients with microscopically confirmed P. vivax mono-infection seeking treatment at Shoa Robit Health Centre during the study period, aged between 1 and 65 years, were recruited and treated with a 25mg/kg chloroquine, administered for three consecutive days. Socio-demographic and selected clinical information were collected using semi structured and pre-tested questionnaire. Blood smears were prepared and examined for checking parasite clearance and/or recurrence of parasitaemia and clinical examination was performed at allfollow-up visits. Haemoglobin (Hgb) was measured using microhematocrit technique. Percentages, frequencies, Kaplan-Meier survival probability analysis and statistical associations were computed. P-value of <0.05 was considered statistically significant.*

Results: *Of the total of eighty seven patients included in the study, four of them were excluded due to P. falciparum infection during the follow up and seven cases were loss to follow-up. From the seventy six study participants, who completed their 28 day follow up, five (6.6%) were with early treatment failure (ETF). Forty four (50.6%) of the study participants were febrile on day of admission and sixty three (72.4%) had history of fever before admission.Geometric mean parasite count of the study participants was 8723.9 /µl. Mean hematocrit value was 35.45%.*

Conclusion: *This study shows probable emergence of chloroquine resistance / treatment failure in P. vivax malaria in Shoa Robit Town, North East, Ethiopia. Regular monitoring and periodic evaluation of the efficacy of this antimalarial drug in systematically selected sentinel sites is recommended.*

Key words: *Plasmodium vivax, Chloroquine, Treatment failure, Shoa Robit*

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LIST OF ACRONYMS AND ABBREVIATIONS

ACT Artemesinin based combination therapy

ACPR Adequate clinical and parasitological response

AL Artemether Lumefantrine

CRPv Chloroquine resistant *P. vivax*

CQ Chloroquine

DCQ Desethylchloroquine

ETF Early treatment failure

HCT Hematocrit

Hgb Haemoglobin

LCF Late clinical failure

LPF Late parasitological failure

LFU Loss to follow-up

MEC Minimal effective concentration

PCT Parasite clearance time

PQ Primaquine

PRR Parasite reduction ratio

SP Sulfadoxine-pyrimethamine

WBC White blood cells

WHO World Health Organization

WTH Withdraw

CHAPTER ONE: INTRODUCTION

1.1Background

From the five *Plasmodium* species known to cause malaria in humans, *P. vivax* is the most widespread. Nearly 2.49 billion of the world's population is living at risk of *P. vivax* infection (1). *Plasmodium vivax* is highly prevalent in Central and South East Asia and it is also common in Latin America and Africa but it is rare in west and central Africa (2). High prevalence of Duffy negativity in Africa has led to a perception that *P. vivax* is absent from many parts of the continent (3). However, there is a report of *P. vivax* infection in Duffy negative people in Africa and other parts of the world recently (4–6). Moreover, *P.vivax* malaria becomes prevalent in previously malaria-free temperate regions, like Greece and central China (7,8).

In Ethiopia, around 52 million people (68%) live in malaria-endemic areas (9). Different from many countries in Africa, *P. falciparum* and *P. vivax* significantly contribute to malaria cases in the country in relative proportions of 60% and 40%, respectively (9–12). Sometime the proportion of *P. vivax* infection increases particularly in low malaria transmission period due to the seasonal drop in *P. falciparum* infection and the relapsing nature of *P. vivax* infection (11).

Chloroquine is the first line drugs for treatment of *P. vivax* malaria in most part of the world, where *P. vivax* is known to be CQ sensitive. A therapeutic dose of 25 mg/kg CQ divided over three days is recommended. However, in areas where *P. vivax* is known to be CQ resistant, Artemesinin based combination therapy (ACT) is an alternative drug (13). In addition the drugs used for the treatment of *P. falciparum* malariaare also effective and can be used for CQ resistant *P.vivax* infections. Drugs like: quinine sulfate plus doxycycline or tetracycline, Atovaquone-proguanil and mefloquine are equally recommended (14). For radical cure, the use of Primaquine (PQ) 0.25 mg/kg/ for 14 days is recommended (15,16).

Current malaria treatment policy in Ethiopia recommends Artemether lumefantrine (AL) as first-line drug for the treatment of uncomplicated falciparummalaria, for mixed infections with vivax malaria and for clinical vivax malaria in the absence of definitive diagnosis. The treatment policy recommends CQ as first-line drug for the treatment of *vivax* malaria. Primaquine is also recommended for radical cure in patients who are not living in malaria endemic areas . Quinine

is the second line drug for the treatment of vivax malaria in the country (9,10,17–19).

Chloroquine is cheap, it can be administered easily in few doses and it is safe for pregnant women and children. Moreover, CQ will be absorbed very rapidly but it will be eliminated slowly as CQ and a Desethyl Chloroquine (DCQ) metabolite. Furthermore, CQ can clear fever and parasitemia caused by *P. vivax* in 3 days (20). Besides, a drug level that can prevent vivax malaria will remain in blood until about day 21to day 35 after the start of treatment (21).

Even though CQ is with such qualities, the emergences of CQ resistant strains of *P. vivax* parasites are challenging the efficacies of this drug. There are reports of decreased CQ susceptibility to vivax malaria throughout the world including Ethiopia.

Resistance to antimalarial drug like CQ is a main public health problem and it hampers the successful control of malaria. Continuous monitoring of CQ efficacy is very important for evidence based decision making concerning the use of this drug in a country and to control the increasing resistance of vivax malaria to CQ (22).

Diagnosis of anti-malarial drug efficacy can be achieved by various techniques including *in vivo* therapeutic efficacy studies*, in vitro* studies, using molecular markers, by molecular genotyping and Pharmacokinetic studies. *In vivo* therapeutic efficacy study is the gold standard and permits measurement of clinical and parasitological efficacy of a drug (13,23,24).

1.2 Statement of the Problem

Plasmodium vivax has not been given emphasis as a public health priority for many years. However, at this time it becomes a global health issue due to its major public health and socio-economic problem for many countries (19,25).

Although, previously vivax malaria was considered to be relatively benign compared to falciparum malaria, nowadays it is causing a range of severe, life-threatening syndromes that are similar to those caused by *P. falciparum* malaria (26–29).

Unlike *P. falciparum,* a single infection of *P. vivax* causes a frequent illness due to multiple relapses after the primary infection. These weaken adults and affect the growth and schooling of children, thus damaging economic development of a country (11,13).

Malaria control strategies are not equally effective for *P.vivax* and *P. falciparum.* This is because the control strategies in *P. vivax* are affected by several factors like: lack of access to reliable diagnosis, the parasite's ability to transmit early in the course of disease, the relapsing nature and a broader geographic range. Therefore, to reduce the burden of disease effective treatment of vivax malaria is imperative and it is one of the most basic and successful global strategies (25,29–31).Effective treatment of malaria is important for both the patients and the community as a whole. Because, it will resolve complication of malaria in patients and slowdown the emergence and spread of drug resistance in the community (11).

Chloroquine is the recommended first-line treatment for *P. vivax* malaria in most parts of the world except in few areas with widespread CQ resistance (16,24,32). However, there is emergence and spread of CQ resistant strains of *P. vivax* in many parts of the world using CQ as first line drug including Ethiopia (33–37).

Antimalarial drug resistance affects the successful control of malaria. Drug resistance will increase disease burden of malaria. There is occurrence of severe vivax malaria in areas with a trait of CQ resistance (28,38–40). Moreover, there is increasing mortality rate due to CQ resistance(41).

The cost for malaria control will be increased due to antimalarial drugs Resistance. Therapeutic failure needs further diagnosis and treatment of malaria, consequently it increases the cost of the health system, loss of working days and absence from school (42).

Ineffective treatment of malaria in public health facilities due to drug resistance might lead to greater dependence of patients on unregulated private health services. This may increase the use of substandard and counterfeit medicines and as a result leading to the emergence and further spread of drug resistance (11,24).

Regular monitoring of CQ efficacy is necessary for early detection of resistance and for timely changing the treatment policy of a country. World Health Organization (WHO) recommends therapeutic efficacy studies to be done for first and second line antimalarial dugs at least every two years. The change of treatment policy is also recommended when the treatment failure rate is above 10% (11,24).

Emergence of CRPv in many countries makes it important to monitor the susceptibility of *P. vivax* to this drug in Ethiopia. To the best of our knowledge, there are only few reports about *P. vivax* drug resistance to CQ in Ethiopia. Moreover, these few reports are good indicators of the emergence and spread of CRPv strains in the country. Therefore, more studies are needed on the degree of resistance of *P. vivax* in other malaria endemic areas of the country to have clear picture of the country wide distribution of this problem. In addition, since there is no information about sensitivity of *P. vivax to* CQ in the study area, the present study is aimed to determine the rate of CQ treatment failure in *P. vivax* in Shoa Robit , one of malaria endemic area of the country.

1.3 Significance of the Study

Chloroquine is still the first line drug for the treatment of *P. vivax* malaria in Ethiopia including the study area. However, few reports are indicating the emergence and spread of CQ resistant *P. vivax* strains in the country. Thus, more studies are needed to know the degree of resistance in malaria endemic regions of the country like Shoa Robit. Thus, the present study will give further information about the status of CQ resistance in the country. Moreover, this study will give evidence for policy makers whether to consider change in the treatment policy to control further expansion of CQ resistance. Furthermore, the study will help as a base line prevalence of treatment failure and associated risk factors for the study area.

CHAPTER TWO: LITERATURE REVIEW

Chloroquine resistant *P. vivax* has been reported in different parts of the world. Most of these reports of CQ resistance are from Central and Southeast Asia, Latin America and Africa. In this review, it is tried to show the different methods used to diagnoses CQ resistance in *P.vivax*, the rate of *P. vivax* resistance to CQ in different parts of the world and risk factors associated with CRPv.

2.1 Diagnostic Methods of Chloroquine Resistance in *Plasmodium vivax*

There are different methods used to monitor antimalarial drug efficacy and drug resistance. For example, in *vivo* tests, in *vitro* tests, use of molecular markers, molecular genotyping and measurement of drug concentrations are the major methods. In *vivo* studies of parasitological and clinical response is the main sources of data for therapeutic efficacy. However, the other therapeutic efficacy studies are used to confirm the result of in *vivo* therapeutic efficacy studies (23,24,43) .

2.1.1 In *vivo* Study

In *vivo* therapeutic efficacy study is a gold standard for monitoring antimalarial drug efficacy. This method requires treatment of patients with *P. vivax* mono infection with a standard dose of CQ and subsequent follow-up of parasitological and clinical outcome for 28 days (24).

In the in vivo therapeutic efficacy study, study participants with an asexual parasitemia level that either increases or 25% of day 0 parasitemia persist to day 2 will be classified as a direct treatment failure. Moreover, a study subject with an asexual parasitemia of any level remaining to day 7 will be classified as an early treatment failure. In addition, an asexual parasitemia that reappears at any time between days 7 and 28 will be classified as a recurrence treatment failure. Furthermore, study participants without any recurrent parasitemia up to day 28 will be classified as having had an infection sensitive to CQ (23). According to the WHO guidelines, treatment outcomes of in *vivo* therapeutic efficacy study will be classified on the basis of parasitological and clinical outcome as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) or an adequate clinical and parasitological response (ACPR) (22).

In addition to recrudescence, there are other factors that cause treatment failure in *P. vivax* malaria after anti-malarial drug treatment. Relapse, reinfection, malabsorpition of the drug, low level of drug in blood and poor drug quality are some of the factors (44,45).

To confirm true treatment failure, patient should be treated with standard CQ regimen. Also confirmation of adequate compliance is required by reliable supervision. Moreover, Direct and early treatment failures can be confirmed by measuring the concentration of CQ and DCQ after the last dose and the concentration should be ≥ 500 ng/ml whole blood. For the recurrence treatment failure the concentration should be ≥ 100 ng/ml whole blood at day of recurrence (23).

2.1.2 In *vitro* Studies

In *vitro* assays are used to monitor drug resistance by measuring the intrinsic sensitivity of parasites to antimalarial drugs. Parasites will be cultivated in vitro with antimalarial drug and it will be observed for inhibition of maturation into schizonts (24). Even though a number of in *vitro* drug sensitivity tests have been reported for *P.vivax*, the best method for determining the drug response of *P. vivax* isolates in *vitro* is not identified (46).

2.1.3 Molecular Marker

Molecular markers are important tools to identify genetic mutation of drug targets or enzymes related to antimalarial drug resistance in the parasite genome. Molecular markers can be used to distinguish between reinfection and recrudescence in therapeutic efficacy studies (24). Even though different studies have been carried out concerning the use of this tools in *P.vivax*, the use of molecular markers for *P. vivax* CQ resistance have not been established clearly. Therefore, monitoring CQ resistance in *P. vivax* through molecular markers is complex (43,47–52) .

2.1.4 Molecular Genotyping

Examination of a parasite genotype can be used to classify recurrent parasitemia of *P. falciparum* infection as a recrudescence or reinfection. Matched genotype of primary parasitemia and recurrent parasitemia is regarded as recrudescence and mismatched genotype of primary parasitemia and recurrent parasitemia is considered to be reinfection (22,24). However, in *P. vivax* infection, genotyping cannot be used to classify recurrent parasitemia as a recrudescence, reinfection or relapse. This is because, more than half of parasites causing relapse do not match with the genotype at baseline. As a result, matched genotype in *P.vivax* malaria may be either a recrudescence or a clonal relapse. Similarly, mismatched primary and recurrent parasitemia may

represent either a new infection or a relapse. Therefore, genotyping cannot be used to differentiate parasitemia originating from *P.vivax* recrudescence, relapse, or reinfection (23,53–55).

2.1.5 Drug Concentration Measurement

The method of determining CQ efficacy in *P.vivax* malaria mainly depend upon remaining levels of CQ in blood for about 28-35 days following therapy and the minimum effective concentration (MEC) of CQ and its major metabolite, desethyl chloroquine (DCQ) in blood at day of recurrence which is, 100 ng/mL of whole blood (44,45). Concentration of antimalarial drug and/or active metabolite(s) can be measured in whole blood, plasma or serum (24).The diagnosis of resistance to CQ involves the measurement of concentration of CQ and DCQ in blood on the day of parasite recurrence. Whether recurrent parasitemia represents recrudescence, relapse or reinfection is not known, if the parasite occurred in the presence of concentrations of drug in blood that can eliminate parasites of the CQ sensitive phenotype, it will be taken as resistance (23,45).

2.2 Prevalence of Chloroquine Resistant Plasmodium vivax

The highest rate of *P. vivax* resistance to CQ was reported from Central and Southeast Asia. In a study conducted in Indonesia in 1998, parasites reappeared in 27 of 52 *P. vivax* patients within 28 days of follow up. In this study, CQ blood levels at the time of recurrent parasitemia was above the MEC in 12 of the 27 individual with recurrent parasitemia and revealed a resistance of 23% (56). In addition, a resistance of 56% was reported from eastern Indonesia in 2002 (57). The highest rate of therapeutic failure, which was 84%, was reported in 2003 in the north-eastern coast of Indonesian Papua (58). Likewise, in a study conducted in Dawei, southern Myanmar in 2003, from 252 study participants included 34% of the participants were with recurrent parasitaemia. In this study, two (0.8%) of the patients with recurrent parasitaemia had CQ concentrations above the MEC and were considered infected with CQ resistant parasites (37). However, the findings of studies conducted in Iran, Afghanistan, Thailand and Pakistan showed susceptibility of *P. vivax* to CQ in the regions (59–62). Except for a recent report of treatment failure in five patients (2.5%) in Thailand. Concentration of CQ and DCQ was above the MEC in one of the patients with treatment failure in this study (63).

Therapeutic efficacy studies in Latin America also suggest the presence of CRPv malaria in the region. Three of the 27 patients (11%) were not cured by CQ in a 28-day follow-up in Colombia in 2001. Measurement of blood CQ and DCQ levels was not performed in this study (64). Furthermore, in a study carried out in Peru, four of 242 patients were with a recurrence of *P. vivax* parasitemia on day 21 and 28, of which, two of these patients had CQ resistant infections based on CQ and DCQ blood levels (65). *Plasmodium vivax* CQ resistance was also confirmed in 11 of 109 subjects (10.1%) who completed a 28 day in vivo drug efficacy test in Brazilian Amazon in 2007 (40). Likewise another study was done in Brazilian Amazon in 2013 and in this study out of 135 subjects, who accomplished the 28-day follow-up, parasitological failure was observed in 7 (5.2%) of patients, in which plasma CQ and DCQ concentrations were above the MEC in all treatment failure cases (66).

There are few data concerning CQ efficacy to *P. vivax* malaria in Africa. A study from Madagascar in 2008 showed a treatment failure rate of 5.1% without measurement of CQ and DCQ concentrations in patients with treatment failure (43). The rest of few reports are from Ethiopia. The first work on the therapeutic efficacy of CQ to *P. vivax* malaria in Ethiopia was done in Debrezeit in 1996 (67). In this study, 225 patients were enrolled and 2% of them failed to respond to the treatment within seven days. Similarly, after ten years another study was conducted in the same area, Debrezeit (33). In this study, 87 subjects were involved and parasitaemia reappeared in 4.6% of the patients on day 28. In addition, a 3.6% prevalence of CRPv malaria was reported in 2009 at Serbo, Jimma zone, south-west Ethiopia (68). Furthermore, a research carried out in Halaba district, South Ethiopia, in 2009 showed the rise of CQ treatment failure to 13% as compared to earlier reports in the country although CQ and DCQ concentration was not measured in patients with treatment failure (34). Another study was also done in Oromo Regional State in 2010. In this study, from 120 patients treated with CQ, resistant reported at day 28 was 2.8% (three patients), after measurement of blood CQ & DQC level (69).

In addition to parasitological clearance, clinical cure is also one component of in vivo therapeutic efficacy study (24). In the study conducted in Halaba district, south Ethiopia, 29% of the study participants were with malaria symptoms such as headache, fever, chilling, and joint pain during the follow-up period even though there were no recurrences of parasitemia in their blood (34). On the other hand, in the study conducted in Serbo none of the patients with treatment failures

had complained malaria symptom (68). Moreover, there may be symptomatic recurrent parasitaemia in some patients. In the study conducted in Debre Zeit, four of the patients with recurrent parasitaemia were with malaria symptoms (33).

Classifications of treatment failure as direct, early, or recurrent treatment failures will provide general useful information on the relative level of resistance in the community. This approach will also be used to assess the relative levels of resistance in individual parasite isolates (23). Parasites penetrating higher drug levels immediately after therapy are more resistant than parasites that penetrate lower drug levels later. The proportion classified as resistant and the median day of recurrence may be used as indicators of the degree of resistance in the parasite population (45) . In therapeutic response study conducted in eastern Indonesia, out of 18 study subjects with treatment failure, three of the study subjects failed treatment on Day 3, with evidence of rising asexual parasitemia (direct treatment failure), and two others were with stable parasitemia to day 7 (early treatment failure). The CQ and DCQ levels on these five subjects were greater than 500 ng/ml of whole blood showing high grade resistance to CQ (57). In addition, in the study conducted in Halaba district, South Ethiopia, with relatively higher recurrent rate, the day of recurrence of parasitemia was day 7 (four patients), day 14 (six patients), and day 21 (one patient) (34). However, in the two studies conducted in Debre Zeit the day of recurrence for all the patients with drug resistance was day 28 (33,69).

Parasite reduction ratio (PRR) is an early marker of therapeutic efficacy, and it has an important function for a rapid assessment of CQ efficacy in *P. vivax.* Parasite reduction ratio at 48 hour is found to be related to the day of recurrence. If the reductions in parasite biomass become lower, the recrudescence of *P. vivax* will occur earlier. In the study conducted in southern Papua, Indonesia the median parasite reduction ratio at 48 h was 7.5/µl (35). Also, relative PRR of the study participants in Halaba district, South Ethiopia, was 10.7/µl (34). However, in a study conducted in Serbo, South West Ethiopia, the mean parasite reduction ratio of the study participants was 227.2/µl after 48 hour (68).

Consistent use of the WHO procedure for determining antimalarial drug efficacy is essential for comparing results within and between countries over time (24). For example, *in vivo* therapeutic efficacy study will require prolonged periods of follow-up time (minimum of 28 days). This is because the CQ remaining in the blood stream for up to 28 days after treatment prevents the

recurrent parasitemia of relapse or reinfection but not recrudescence. Therefore, a recurrent parasitemia whatever the source will not occur within 28 days of standard CQ therapy unless the parasite is resistant strain (45). This shows how the numbers of follow up days are important in the determination of CQ efficacy in *P.vivax.* However, some of the previous studies used short follow up days, less than 28 days. Besides, measurement of blood chloroquine level was not performed in some of the studies conducted previously (34,43,64,67).

2.3 Risk Factors for Chloroquine Resistance in *Plasmodium vivax*

In addition to a random nature of genetic emergence of drug resistance, there are some risk factors that contribute to the emergence of CQ resistance in *P.vivax* like age, number of parasites exposed to the drug, poor drug quality, poor adherence, vomiting of a drug and presence of other antimalarial drugs in the blood are some of the risk factors of resistance (70). Moreover, differences in diet or in rate of CQ absorption or metabolism could contribute to clearance of a parasite (71). However, there are few data concerning risk factors associated with CRPv.

The rate of recrudescence is higher mostly in young children, who have less immunity and frequently greater parasite burden (72). This is because, immunity has a capacity to reduce the emergence and spread of drug resistance by its ant parasitic effect (24). The majority of treatment failures recorded in the earlier studies are occurred in young children (33–35,37,65,68). Besides, in a study carried out in Thailand, children <5 years old were at greater risk of treatment failure than older patients (73). Moreover, in the study conducted in southern Papua, Indonesia, infants were more likely to suffer an early treatment failure than adults (35). However, in a study done in Brazil, there was no association between age and treatment failure (66).

There is a report of highest parasite density in children less than 5 years as compared to other age groups (68) . Higher parasitemia on day 0 was also found to be a risk factor for CQ resistance in *P. vivax* (66).Moreover, high baseline parasite density found to be a predictor of CQ resistance in patients with falciparum malaria (74). Furthermore, an increase in parasite density on day of parasite reappearance compared to enrolment day in cases of treatment failure was reported (64,68). On the contrary, in the study done in Halaba district, South Ethiopia, the load of parasitaemia on the day of treatment failure was lower than the day of admission (34).

A lower initial hemoglobin concentration was found to be associated with recurrence in Afghanistan (75). Moreover, in the study conducted in Serbo, South West Ethiopia, there was recovery of haemoglobin among all study participants with ACPR. Though in patients with treatment failure, there was no difference in Hgb level on day 0 and on day of recurrence (68).Even though in a study conducted in Brazil, hemoglobin level, gender and body temperature were not found to be associated with resistance (66). However, there is a report of body temperature ≥38° as a factor contributing to delay in parasite clearance in uncomplicated falciparum malaria (74).

CHAPTER: THREE OBJECTIVES

3.1. General Objective

- The general objective of this study was to assess the therapeutic efficacy of chloroquine for the treatment of uncomplicated *Plasmodium vivax* malaria in Shoa Robit, north east Ethiopia.

3.2. Specific Objectives

- To measure the clinical and parasitological efficacy of chloroquine in patients by determining the proportion with early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) or an adequate clinical and parasitological response (ACPR) as indicators of efficacy.

- To determine the HCT levels of the study participants on day 0, 28 and on day of recurrence of parasitemia.

- To assess possible risk factors associated with *P. vivax* CQ resistance.

CHAPTER FOUR: METHODOLOGY

4.1. Study Area and Period

The study was conducted in Shoa Robit Health Centre found in Shoa Robit Town, Kewet woreda, north shoa zone, north-east Ethiopia. The town is located at 225 Kms northeast of Addis Ababa, in the Amhara Regional State at an average elevation of about 1,280 meters above sea level. 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 42,208. The area 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 Shoa Robit and reported throughout the year (11,76). The study was conducted from October 2013 to February 2014.

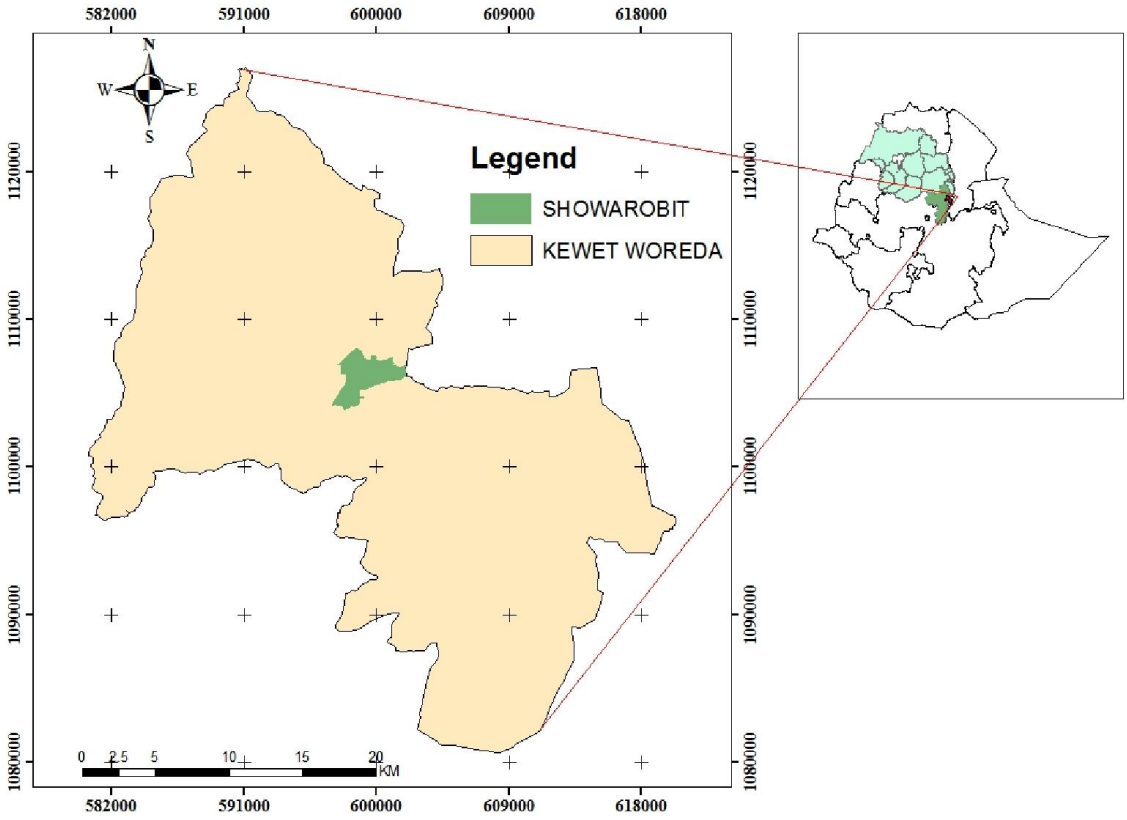

Figure 1 Map of the Study area.

4.2. Study Design

The study was a one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment of uncomplicated *P. vivax* malaria. People with *P. vivax* mono infection who met the study inclusion criteria were enrolled, treated with CQ and monitored for 28 days. The follow up included a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. Based on the results of these assessments, the patients were classified as having therapeutic failure (early or late) or an adequate response. The proportion of patients with therapeutic failure during the follow-up period was used to estimate the efficacy of CQ (22).

4.3 Population

4.3.1 Source Population

Clinically suspected individuals with symptoms suggestive of malaria illness and seeking treatment at Shoa Robit Health Centre during the study period were the source population.

4.3.2 Study Population

Those patients who had confirmed *P. vivax* mono-infection on thick and thin blood film preparations and who fulfilled the inclusion criteria set by WHO and seeking treatment at Shoa Robit Health Centre during the study period were the study population.

4.4. Sample Size and Sampling Techniques

4.4.1. Sample Size

There was no earlier report of CRPv in the study area and the sample size was calculated based on the expected proportion of *P. vivax* treatment failure with CQ in the study population. The sample size was calculated with an expected treatment failure rate of 5%, a confidence level of 95% and a precision level of 5% using the formula, N = (Z/d)2 P (1-P) and with 20% expected loss to follow-up over 28 days (22). Therefore, the required sample size for this study was 87 individuals.

4.4.2. Sampling Techniques

Individuals attending the Health Centre at the time of data collection and who fulfilled the inclusion criteria were enrolled using convenient sampling procedure.

4.5. Inclusion and Exclusion Criteria

4.5.1 Inclusion Criteria

The inclusion criteria were; 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 h before recruitment, ability to swallow oral medication, ability and willingness to comply with the study for the duration of the study and to comply with the study visit schedule, informed consent from the patient or from a parent or guardian in the case of children (22).

4.5.2 Exclusion Criteria

Presence of clinical condition due to vivax malaria (coma, severe anaemia) requiring hospitalization, regular medication, which might interfere with antimalarial pharmacokinetics, pregnancy and breastfeeding (22).

4.6 Measurement (Variables)

4.6.1 Dependent Variables

Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria.

4.6.2 Independent Variables

- Sex
- Age
- Parasite density
- Parasite clearance time
- HCT level
- Clinical symptoms
- Temperature

4.7 Drug quality analysis

The quality of drug used in the study (Chloroquine phosphate 250 mg, batch number KT 1474, Cipla LTD India), was tested for standard concentration using the standard procedures recommended by united state pharmacopoeia at the National Laboratory of Ethiopian Food, Medicine and Health care Administration & Control Authority (EFMHACA). Chloroquine phosphate tablets contain 93%-97% of the labeled amount of Chloroquine phosphate. The result of this test showed the content to be 105.7%. Therefore, the batch of drug used was confirmed to fulfill international specification set for the test.

4.8 Patient Enrolment

Blood films were taken at initial presentation of patients. Once a positive *P. vivax* infection was identified by microscopy, the patients were screened out for study eligibility criteria. All women aged 13–49 years underwent a urine pregnancy test and those testing positive were excluded from the study. Patients confirmed to fulfill the inclusion criteria, and volunteer to participate in the study were enrolled in the study. The *in vivo* test was performed according to the WHO protocol (22). Physical examination including axillary temperature measurement was performed and demographic information was taken at baseline (day 0) before treatment.

4.9. Treatment

Based on the treatment guidelines of the Federal Ministry of Health of Ethiopia (FMOH), the patients were treated with a 25mg/kg CQ (batch number KT 1474 Cipla LTD India), administered for 3 consecutive days(10 mg/kg on days 0 and 1, and 5 mg/kg on day 2). All doses were administered under direct observation by a member of the research team. Study participants were checked for vomiting for 30 minute after ingestion of the drug. The study participants were advised not to take other drugs, except for patients with axillary temperature > 37.5°C who were treated with paracetamol (10 mg/kg). Patients were told to return for follow-up on Days 1, 2, 3, 7, 14, 21 and 28. Patients were also encouraged to come back to the Health Centre if they feel ill at any time during the follow-up period for clinical and parasitological examination.

4.10. Follow-up

A successive monitoring of parasitological and clinical responses was conducted for 28 days. Thick and thin blood smears were prepared and examined for checking parasite clearance and/or recurrence of parasitaemia at all follow-up visits. Parasite clearance time (PCT), defined as the time from the start of CQ treatment until blood films become negative, was determined. Haemoglobin measurement was made on day 0 and 28 during follow-up and on day of recurrence of parasitaemia using hematocrit tube. Patients who did not come to the Health Centre on the scheduled date were asked to come and tried to retain them in the study. Those patients who failed to respond to CQ were retreated with quinine (10 mg base/Kg), the second line drug according to the national treatment guidelines. All re-treatments were done under supervision.

The patients were followed for any complain and released after the blood films of the cases were checked for parasite clearance.

4.11. Parasite Detection

Parasites were identified by using microscopic observation of the parasite's morphology using thick and thin blood smears prepared on day of enrolment (Day 0). In addition, thick and thin blood films were prepared and examined at subsequent visits. After fixing the thin film in methanol, both the thin and thick smears were stained with Giemsa (3%, pH 7.2) for 30 minutes. A fresh Giemsa stain dilution was prepared for a day. The thin and the thick films were examined under oil immersion objective to identify the parasite species and to determine the parasite density. The thick blood smear was used to count the numbers of asexual parasites and white blood cells in a limited number of microscopic fields. *Plasmodium vivax* asexual stages were counted against 200 white blood cells (WBC), assuming the average total WBC count of 8,000/µl. Parasite density, expressed as the number of asexual parasites per µl of blood, was calculated by dividing the number of asexual parasites by the number of WBC counted and then multiplying by an assumed WBC density (8000 per µl) (77).

$$\text{Parasite density (per µl)} = \frac{\text{number of parasites counted} \times (8000)}{\text{Number of leukocytes counted (200)}}$$

When the number of asexual parasites was less than 10 per 200 WBC in follow-up smears, counting was done against 500 WBC. A blood slide was considered negative when examination of 1000 WBC revealed no asexual parasites. The presence of gametocytes on enrolment or follow-up slide was noted. Hundred fields of the thick film were examined to exclude mixed infections; in case of any doubt, the thin film was examined for confirmation.

Parasite reduction ratio from day of admission to day 2 (after 48 hours), was evaluated using the formula $\tau = Po/P2$ (where Po is parasite count on day 0 and P2 parasite count on day 2) (78).

4.12. Data quality Control

The questionnaire was prepared in English and translated to Amharic then translated back to English to confirm the correctness of the translation by language expert. Standard Operating

Procedure (SOPs) was prepared according to different text books and manuals (annex-3). It was strictly followed during reagent preparation, specimen collection and processing and microscopic examination. Experienced laboratory technologists examined each blood smear. All *P. vivax* positive slides on day of admission, 10% of negative slides picked randomly from slides prepared during follow-up and all slides on day of recurrence were re-examined by senior laboratory technologist and the results were concordant.

4.13. Endpoints

All patients were classified as having early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response. Study participants were considered to have cleared parasitaemia if there were at least two sequential negative smears. The day on which the first such negative smear was observed was defined as the day of clearance.

4.14. Data Analysis

Data collected from the *in vivo* study was double entered and interpreted using a Program designed by the Global Malaria Program which runs on Excel and which ensure that the results of the study are interpreted with the methods recommended by WHO. Kaplan-Meier survival probability analysis was used to evaluate treatment outcome of study participants during follow-up period. The analysis included the cumulative incidence of success and failure rates at day 28. SPSS software (version 20) was used for the other analysis. Haemoglobin recovery of patients with ACPR was compared using Wilcoxon Signed Ranks Test. Also proportion of anaemic cases on day of admission and day28 was evaluated using chi-square goodness-of-fit test (Pearson's chi-square goodness-of-fit test). In non-normally distributed data (age), median was used to measure the central tendency. Parasite count and parasite reduction ratio was analyzed using geometric mean. Relationship between parasite load with age of study participants on day of admission and parasite clearance time with parasite density at enrolment was compared using Pearson's correlation. Based on purposeful selection of variables in logistic regression, preliminary bivariate analysis for each independent variable was performed and those variables with P-value of less than 0.25 at the bivariate regression were then selected to multivariate analysis model. In all analysis, p-values < 0.05 was considered significant (22).

4.15 Operational Definition of Terms

- ✓ Day 0 - The day on which the patient is enrolled and receives the first dose of medicine (22)
- ✓ Treatment failure **-** An inability to clear malarial parasitaemia or resolve clinical symptoms despite administration of an antimalarial medicine (24).
- ✓ Recurrent parasitaemia - The presence of new parasite of unknown origin in the blood (79).
- ✓ Early treatment failure - Danger signs or severe malaria on day 1 to 3 in the presence of parasitaemia; parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; parasitaemia on day 3 with axillary temperature ≥ 37.5 ºC; parasitaemia on day 3 ≥ 25% of count on day 0.
- ✓ Late clinical failure - Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28; presence of parasitaemia on any day between day 4 and day 28 with axillary temperature ≥ 37.5 ºC or history of fever.
- ✓ Late parasitological failure - presence of parasitaemia on any day between day 7 and day 28 and axillary temperature < 37.5 ºC.
- ✓ Adequate clinical and parasitological response **-** Absence of parasitaemia on day 28, irrespective of axillary temperature (22).

4.16. Ethical Clearance

Ethical clearance was obtained from Jimma University, Ethical review board. Official request letter was submitted to Shoa Robit Town administration health office and permission was obtained to conduct the research. The objectives as well as the nature of the study was explained to the participants and written informed consent was obtained from each patient or guardian parents, in cases when the study participants were younger than 18 year.

CHAPTER FIVE: RESULTS

During the study period, a total of 3076 patients were examined for malaria infection and 296 (9.6 %) of them were positive for malaria by blood film microscopy. From the positive cases, 36 % (107/296) were with *P. vivax* mono-infection. Eighty seven of the patients with *P. vivax* mono-infection, who met the inclusion criteria set by WHO (22), were enrolled in the study.

5.1. Demographic and Clinical Characteristics of the Study Participants

The demographic and clinical features of the study participants are shown below (Table 1). Median age of the study population was 20 years (1 year to 65 years), and the majority (71.3%, n = 62) of the study participants were males. A third of the study participants (33.3%, n = 29) were children <15 years. Duration of their illness (mean ± SD) before enrolment was 3.99 ± 2.11 days. 73.6% (n=64) of the study participants were from urban and 26.4% (23) were from rural. Based on data from questionnaire, none of the study participants took antimalarial drug before recruitment. Of the total 87 study participants included in the study, 11 were excluded during the 28 days follow-up for different reasons. Seven of them were loss to follow-up (LFU) on day 3(two cases), on day 7(two cases) and on day 14 (three cases) and four of them were withdrawn (WTH) from the study due to *P. falciparum* co-infection on day 2 (one case), on day 7 (two cases) and on day 14 (one case) and re-treated with AL. Five of the study participants were with recurrent parasitemia and were re-treated with quinine. At the end, seventy one of the study participants completed the 28 days follow up successfully (Table 2).

Table 1. Summary of Patients' characteristics at baseline, Shoa Robit Town , North Eastern Ethiopia, October 2013 to February 2014.

Year	2013/14
Months	Oct-Feb
Number of patients	87
Residence	
Urban	73.6%
Rural	26.4%
Ratio male/female	62/25
Age group (years)	
under 5	13
5 to 15	16
Adults	58
Age (years)	
Median	20
range (min-max)	1-65
Hematocrit (%), day 0	
mean (sd) range (min-max)	35.45(5.1) 21–46
Anemic at day 0	
Under 5	10
5 to 15	9
Adults	21
Duration of illness (days) mean (sd)	3.99 (2.1)
Temperature (°C), day 0	
mean temperature (sd)	37.3 (0.5)
range (min-max)	36-38
Parasitemia (µl), day 0	

mean (geometric) parasitemia	8724
range (min-max)	440-29500

Symptoms such as fever, headache, vomiting, cough, diarrhoea and joint pain were reported by the patients at the time of recruitment. Among these, the major were fever (75.7%) and headache (63.1%). Vomiting, diarrhea and joint pain were also reported in 9.1% ,4.6% and 9.2% of the study subjects on day of admission respectively. About half of the study participants, 50.6% (n = 44) were febrile on day of admission, (had axillary temperature ≥37.5°C) and 72.4% (n = 63) had history of fever in the previous 48 hours before admission. None of the study participants were with severe malaria in the follow up days.

Geometric mean parasite count of the study participants at baseline was 8724 /µl (95% CI = 440 – 29500). Among the study population, age and parasite density at day 0 were observed to have a medium negative correlation (r = -0.355, significant at p = 0.01) (Figure 2).

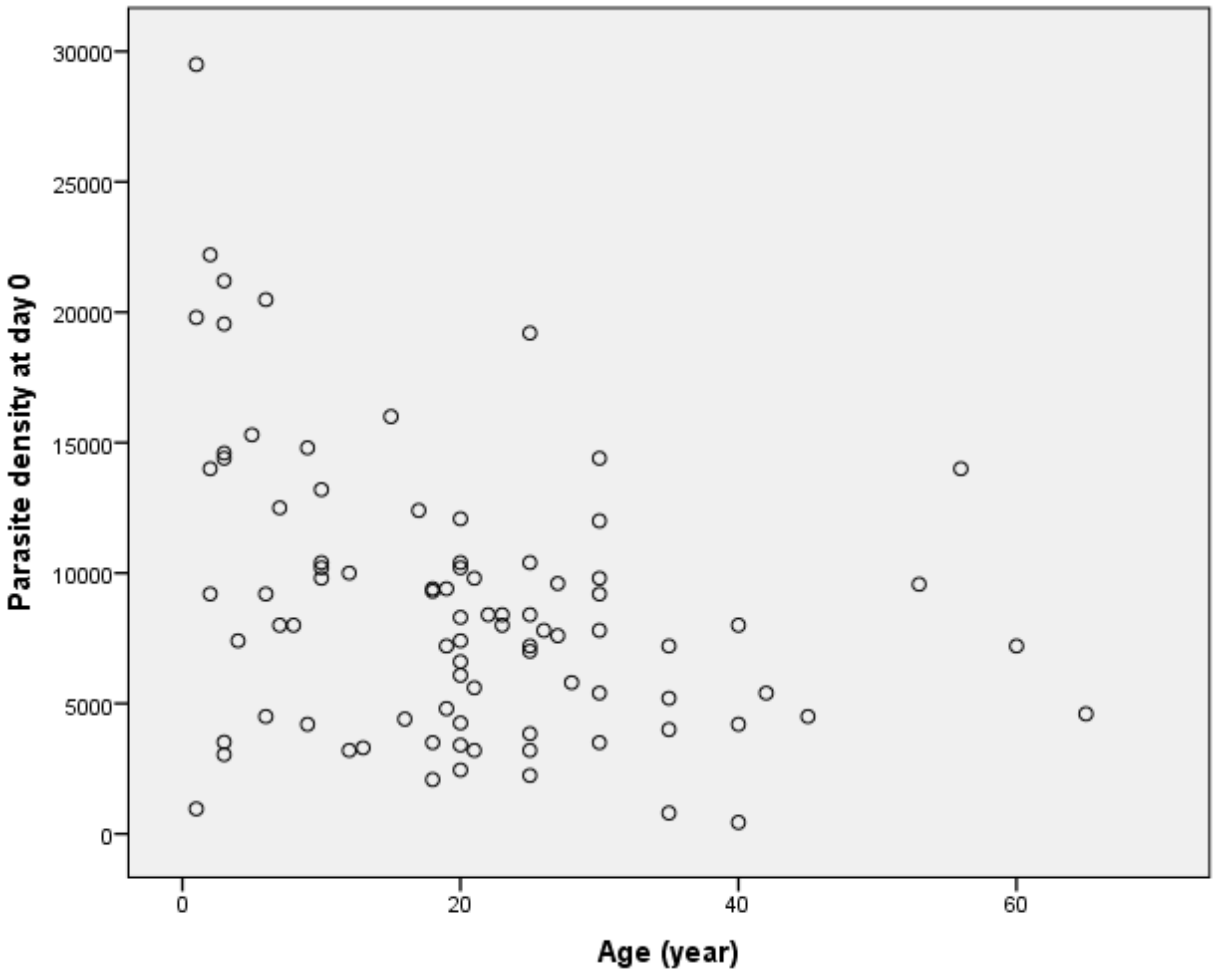

Figure 2 Relationship between age and parasite count at day of admission of study participants in Shoa Robit Town, north eastern Ethiopia, October 2013 to February 2014.

5.2. Treatment Response

Parasite clearance was achieved within 48 hours in 93.1% (n = 81) of the study participants and full parasite clearance was achieved on day 3 in all cases. The mean parasite reduction ratio was 34.14 on day 2. Gametocytes were detected in 52.9% (n = 46) of the study subjects' blood films on days of admission. In all cases, gametocyte cleared within 3days and did not re-appear until the end of the follow-up period.

Table 2. Summary of treatment outcome of the study participants in Shoa Robit Town, north eastern Ethiopia, October 2013 to February 2014.

	number	%	lower 95%CI	upper 95% CI
ETF	0	0.0	0.0	4.7
LCF	0	0.0	0.0	4.7
LPF	5	6.6	2.2	14.7
ACPR	71	93.4	85.3	97.8
Total patients per protocol	76			
WTH	4			
LFU	7			
Total patients LFU/WTH	11	12.6		
Total patients at baseline	87			
Day 3 parasite clearance				
	number	%	lower 95%CI	upper 95% CI
Day 3 positive	0	0.0	0.0	4.2
Total analysis at day 3	86			

ETF-early treatment failure, LCF-late clinical failure , LPF- late parasitological failure, ACPR- Adequate clinical and parasitological response.

Among the 87 patients treated with CQ, five (6.6%) were with LPF on day 7 (one case), on day 14 (three cases) and on day 21(one case). Seventy one (93.4%) of the study participants were with ACPR. There was no ETF or LCF in the present study (Table 2). The cumulative incidence of success and failure rates at day 28 was 93.4% (95% CI= 85.3-97.8) and 6.6 %(95% CI= 2.2-14.7), respectively (figure 3). In all patients with treatment failure, parasite count decreased from day of admission to day of parasite recurrence. All of the study participants with treatment failure were without complains of malaria symptoms except for the recurrence of parasitemia.

Four of the patients with treatment failure cleared their parasitemia within 48 hours, while one of the patient with treatment failure cleared parasitemia on day3

Figure 3 Kaplan-Meier survival curve of the study in Shoa Robit Town , North Eastern Ethiopia October 2013 to February 2014.

5.3. Parasite Clearance Time

The mean parasite clearance time of the study participants was 1.82 days. Moreover, the median parasite clearance time was day 2, where 28.7% (n = 25) were free of parasites on Day 1, 60.9% (n = 53) cleared on Day 2, and 10.3% (n = 9) cleared on Day 3. There was no significant correlation between parasite clearance time and parasite density at enrolment (Pearson correlation, $r = 0.134$, $P = 0.215$).

5.4. Hematocrit Determination

The mean hematocrit of the study participants was 35.45% (21%–46%). Moreover the hematocrit on day of admission and day 28 were significantly different (Wilcoxon Signed Ranks Test, p= 0). (Figure 4)

In addition, chi-square goodness-of-fit test (Pearson's chi-square goodness-of-fit test) was used to determine the proportion of anemic cases on day of admission and on day 28.The proportion of anemic cases on day of admission was not significantly different ($\chi 2 = 0.56$, $p = 0.45$). However, on day 28, the number of anemic cases differed significantly ($\chi 2 = 39.56$, $p = 0.00$). Conversely, in three of the patients with treatment failure, there was no change in the HCT on day of enrolment and on day of recurrence. Furthermore, in two of the study participants with treatment failure, the level of HCT decreased on day of recurrence compared to the day of admission.

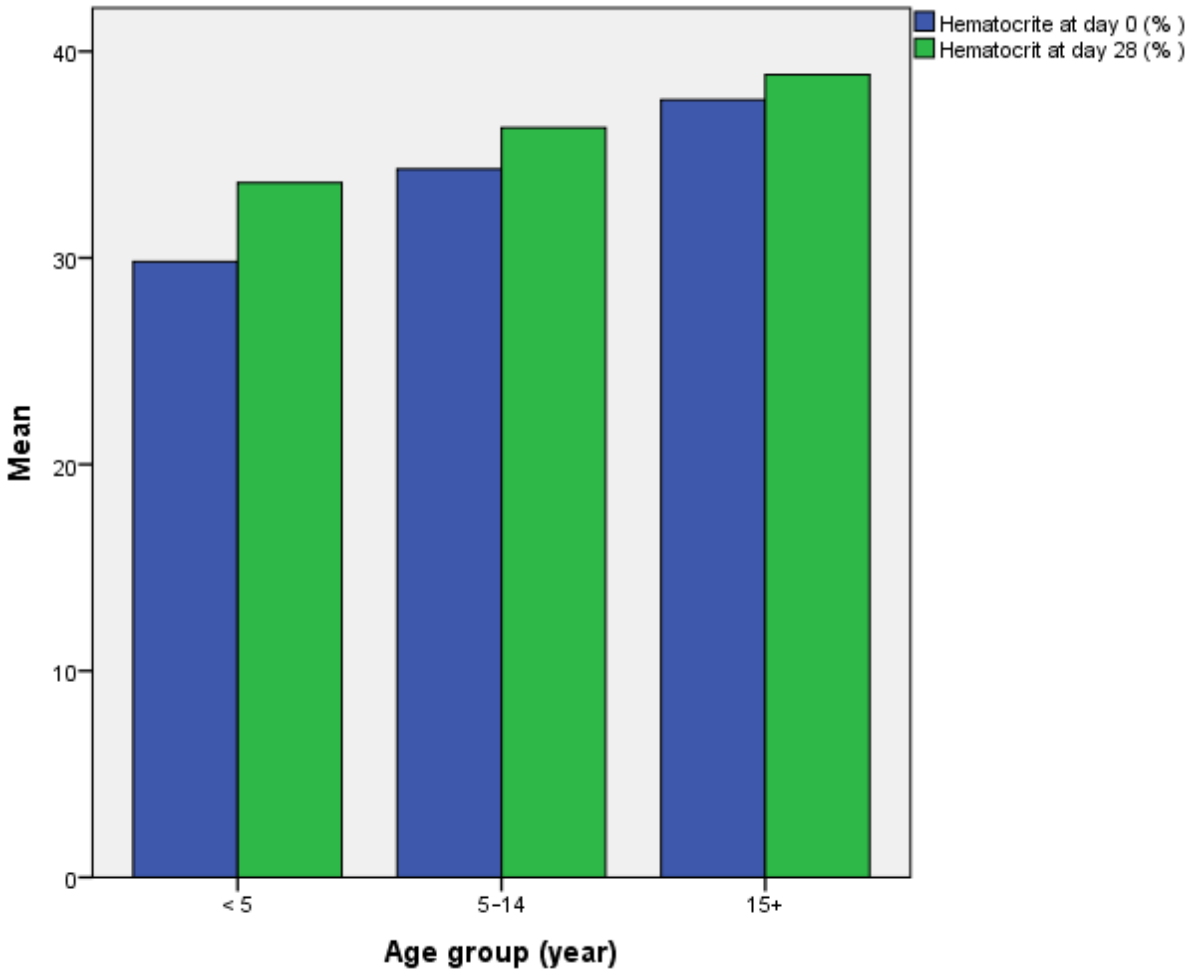

Figure 4 Hematocrit recoveries among study participants with adequate clinical and parasitological response (n = 71) in different age groups in Shoa Robit town, North East Ethiopia, October 2013 to February 2014.

5.5. Association between Treatment Failure and Risk Factors.

To identify the factors associated with treatment failure, binary logistic regression was performed. Age, sex, HCT level, asexual parasite density, temperature, residence, income and level of education were used as predictor variables. In the binary logistic regression, only asexual parasite density showed a statistically significant association with treatment failure (OR=10.261, 95% CI: 1.088-96.736, P-value = 0.042) (Table 3).

Table 3 Binary logistic regression Estimate of Predictor Variables on Prevalence of Chloroquine treatment failure in Shoa Robit Town, North East Ethiopia, October 2013 to February 2014.

	OR	Lower	Upper	P-value
Age	0.116	0.012	1.090	0.060
Sex	1417	0.000		0.998
HCT level	2307	0.000		0.997
Parasite density	10.260	1.088	96.730	0.042
Temperature	0.667	0.106	4.201	0.666
Residence	0.000	0.000		0.998
Income	0.232	0.033	1.552	0.132
Level of education	2.189	0.234	20.509	0.493
Constant	0.182			0.027

Variables with P-value of less than 0.25 at preliminary bivariate analysis were selected as candidate for multivariate analysis. Accordingly; Age, asexual parasite density and income which were found to have a P-value of less than 0.25 in initial bivariate analysis, were selected as candidate variables and entered into multivariate logistic regression analysis. In multivariate logistic regression, Age, asexual parasite density and income did not show a statistically significant association with treatment failure (Table 4).

Table 4 Multivariate logistic regression Estimate of Predictor Variables on Prevalence of Chloroquine treatment failure in Shoa Robit Town, North East Ethiopia, October 2013 to February 2014.

	AOR	Lower	Upper	P-value
Age	0.226	0.021	2.439	0.221
Parasite density	6.221	0.578	66.945	0.132
Income	0.245	0.031	1.936	0.182
Constant	0.118			0.100

CHAPTER SIX: DISCUSSION

Chloroquine resistant *P.vivax* malaria is emerging in different parts of the world. Several studies conducted in various parts of the world, including Africa, treatment failure rate ranging from 0% to 84% is reported (40,43,56–59,65,69). Following the emergence of CQ resistance, some countries changed treatment regimen of vivax malaria from CQ to ACT. Chloroquine is the recommended anti-malaria drug in Ethiopia for treatment of *P. vivax* malaria infection. However, reports of emergence of CRPv are appearing in some parts of the country (33,34,67,68).

In support of the previous reports, this study also confirmed CQ treatment failure for vivax malaria in Shoa Robit. The present study, revealed a treatment failure rate of 6.6% (n = 5). This finding is comparable with the previous reports from Debrezeit (33) and Serbo (68) in Ethiopia, in which treatment failure rates of 4.6% and 3.6% were reported, respectively. A study from Brazil (66) and Madagascar (43) also reported comparable magnitude of treatment failure 5.2% and 5.1% respectively.

The magnitude of resistance observed in this study is much lower compared to the 34% resistance in Myanmar (37), 56% reported from Eastern Indonesia (57) and 84% from north-eastern cost of Indonesia (58) , where CRPv is common. On the other hand, the treatment failure rate in this study is higher than findings in Iran, Afghanistan, Thailand, Pakistan and Peru (59–63,65).

These differences in the rate of treatment failure may be because of variation of methods used, the demographic characteristics of the population studied, the geographical difference in the parasites strain and differences in time the studies were conducted.

Plasmodium vivax that recurs earlier immediately after therapy are more resistant than parasites that recur later. Moreover, the median day of recurrence may indicate the degree of resistance in the parasite population (45). In our study, there is no early treatment failure and all the treatment failures appeared on days between 7 and 28. The days of recurrence in this study were day 7(one

case), day 14 (three cases) and day 21 (one case). This finding was similar with the study finding in Halaba district, South Ethiopia (34) ; however, different from the two study findings in Debre Zeit, in which the day of recurrence for all the study participants with drug resistance was day 28 (33,69). The difference on the day of recurrence indicates the presence of variation in the degree of resistance in different parts of Ethiopia.

Our finding indicates that the mean parasite clearance time was 1.82 days, which is comparable with one of the studies done in Iran (80) but lower than the other (59). This difference in parasite clearance time may be due to variation in the susceptibility of *P. vivax* malaria to CQ in different areas. In the present study, there was no significant association between parasite clearance time and parasite density at base line. This is similar with the study conducted in Iran (59).

Five of the study subjects with treatment failure were without complain of malaria symptom except, for the recurrence of parasitaemia. This finding is in line with the study finding in Serbo, in which none of the patients with treatment failure had complained of malaria symptom (68). However, this finding is different from a study finding in Debre Zeit, in which four of the patients with recurrent parasitaemia were symptomatic (33).

Treatment failure of *P. vivax* malaria after anti-malarial drug treatment may be because of relapse of the parasite, reinfection, recrudescence, malabsorpition of the drug, poor drug quality and low level of drug in blood (44,45). In the present study, the possible causes of the treatment failure could be either recrudescence or malabsorption of the drug. Because most of the treatment failures detected in this study were at the early stage (before Day 17) of the follow-up, (Day 7, and 14, except one case on day 21), on these days the blood level of CQ and DCQ do not drop to level below the MEC (45). Accordingly, the concentration of the drug at this time can prevent the recurrence of parasitemia of relapse or reinfection. Even though, CQ blood concentration data at the day of treatment failure was not available to confirm resistance, the patients were treated with a standard 25 mg/kg CQ regimen and all drug doses were administered under direct observation by the research group and none of the study subjects had recurrent vomiting. These situations are expected to reduce the risk of treatment failure attributable to poor dosing.

The study participants with treatment failure were children at age of 1,3,8,9 and 17 years. This is in agreement with earlier reports, in which treatment failures were observed in children

Findings from this study depicted that higher parasitemia on day 0 was not statistically associated with treatment failure. This result is in contrast with findings from the study conducted in Brazil (66). The load of parasitaemia on the day of treatment failure was lower than the day of admission. This is similar with the study done in Halaba district, South Ethiopia (34) and different from the studies conducted in Colombia and Serbo in South west Ethiopia (64,68).

In this study the same batch of standard CQ was used. Moreover, all study participants were given a directly observed treatment and none of them vomited the drug. None of the study participant responded to take antimalarial drugs before enrolment. Therefore, poor adherence, vomiting of a drug and presence of other antimalarial drugs in the blood were not studied as risk factor.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

This study showed that there is a 6.6% prevalence of CQ treatment failure in Shoa Robit, confirming the emergence of CRPv strains. The days of treatment failure in the study were day 7 (one case), day 14(three cases) and day 21(one case). The mean parasite clearance time of the study subjects was 1.82 days. There was no significant correlation between parasite clearance time and parasite density at base line. There was hemoglobin recovery in patients with ACPR. However, in three of the patients with treatment failure there was no change in the HCT value on day of enrolment and on day of recurrence. Moreover, in two of the study participants with treatment failure the level of HCT decreased on day of recurrence compared to the day of admission. Age, sex, HCT level, asexual parasite density and temperature did not show a statistically significant association with the prevalence of treatment failure.

7.2. Recommendation and future direction

Further spread of resistant strains of *P. vivax* should be monitored in Shoa Robit. It is also very important to conduct therapeutic efficacy studies on vivax malaria in selected sentinel surveillance sites as representative of the various epidemiological level for malaria for current estimate of the burden. Moreover, it is recommended to regularly monitor therapeutic efficacy of CQ to detect further development and spread of resistance to make timely national drug policy changes.

LIMITATION OF THE STUDY

The main limitation of this study is the absence of Chloroquine and Desethylchloroquine blood concentration data at the day of treatment failure. This was not done because of budget constrain.

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ANNEXES

ANNEX 1. INFORMATION SHEET AND CONSENT FORM(English version).

Title of the Research Project

Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria in Shoa Robitnorth eastern Ethiopia.

Name of Principal Investigator: Seble Seifu

Name of the Organization: Jimma University, College of public Health and Medical Sciences

Name of the Sponsor: Jimma University

Information sheet and consent form prepared for study participants attending Shoa Robit Health Center who are going to participate in Research Project.

Introduction

This information sheet and consent form is prepared with the aim of determining the Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria in Shoa Robitnorth eastern Ethiopia 2013- 2014 and to forward the possible solutions in controlling the spread of resistance strain to concerned bodies. The research group includes the principal investigator, and advisors from Jimma University.

Research description

This is a study focusing on detection of treatment failure for chloroquine treatment of *Plasmodium vivax* malaria in Shoa Robit Health Centre. Antimalarial drug resistance is a main public health problem as it hampers the successful control of malaria. Unless antimalarial drug resistance is inspected regularly, the disease burden and the economic costs of malaria will rise radically. Therefore monitoring drug resistance is essential for timely changing to treatment policy.

Chloroquine is the recommended first-line treatment for *Plasmodium vivax* in Ethiopia. But there are few reports about *P. vivax* drug resistance, to CQ in Ethiopia. These few reports are indicator of the emergence and spread of chloroquine resistant *P. vivax* strains in the country.

the main aim of this study is to detect and assess the prevalence of treatment failure. This will help to know the degree of resistance of *P.vivax* in the study areas and to consider planning program for these problem.

The laboratory examination requires collection of blood sample by employing standard procedure.

Risks

There will be no foreseeable risks to you except that you may fill discomfort while collecting blood sample.

Benefits

There will be no special benefits to you except the laboratory test results. The laboratory findings would be prudently used in conjunction with the clinical Findings to initiate appropriate treatment for your medical problem.

Confidentiality

Privacy during interviewing and confidentiality of information are guaranteed. Laboratory sample will be collected and tested confidentially and result will be known by only the research team.

Compensation

No compensation will be available for your time and any inconvenience but we are very grateful to you for taking part in this study.

Contacts

If you have any questions now please feel free to ask me. In case you have any later on, you can contact the principal investigator, Seble Seifu, on the telephone number - 0913517346.

Mr AhmedZeynudin telephone number 0911733132 and Mr. Endalew Zemene telephone number 0912071295.

If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson of the Institutional Review Board, Jimma University School of Public Health and Medical sciences on the telephone number 0471120945.

CONSENT FORM

I am requested to participate in the study by being informed of the objective of the study, which is to determine the Therapeutic efficacy of chloroquine for treatment of Plasmodium vivax malaria in Shoa Robit, northeastern Ethiopia.

Because I believe that the results of this study have importance to be used as a guide for the prevention and/or control of further expansion of resistance against chloroquine in Shoa Robit and the country as a whole, I have agreed voluntarily to provide the necessary information answering all the questions included on the questionnaire; without requesting any costs from the researcher.

Signature of participant: _____ date _____

INFORMATION SHEETAND CONSENT FORM AMHARIC VERSION

የጥናትመረጃመስጫቅፅ

ጤናይሰጥልኝ

የተመራማሪ ስም፡ሰብለ ሰቭዊ

የአማካሪዎች ስም፡አህመታ ዘቭት ፡ት እና እንዳለውዘመት

ተቋም፡የጅማዩኒርሲቲየህክምናናጤናሣይንስኮሌጅየህክም ላብራቶሪሣይንስእናፓቶሎጂትምህርትክፍል፡፡

ስፖንሰር፡ጅማዩኒቨርሲቲ

የጥናትርዕስ፡በሸዋሮቢፔ ጸተማ ጎ፣ ኃቢቨ ባወባ መ ሀቲፔ(ክሎሮጺት ወዋሽትፔ) ባተተረ ጎ ፔ (THERAPEUTIC EFFICACY OF CHLOROQUINE FOR TREATMENT OF *PLASMODIUM VIVAX* MALARIA IN SHOA ROBIT,NORTH EASTERNETHIOPIA)

ማብራሪያ፡ይህየጥናትመረጃየተዘጋጀውበሸዋሮቢፔ ጸተማ ጎ፣ ኃቢቨ ለህክም ለመን፡ እና ለተለያዩምርመራዎችለመጡበጥናትላይለሚሳተፉሰዎችየተዘጋጀነው፡፡

መግቢያ፡ይህየጥናትመረጃየተዘጋጀውየወባመዳኒት **መላመታት** እና ስር ቱት በሸዋሮቢፔ በሚ ገዙ ባወባ በሽተኣኖ ላቭ ለማጥናትአልሞነው፡፡ ጸጎ ቱ ባሚ ገፀው መረ ባዛ ራሳቭቱት ባመላመታ ስርጭትለመቆጣጠርእናየመፍትሔእርምጃለመውሠድያገለግላል፡፡በጥናቱምላይአንድየጥናቱዋናተመራማሪ፡፡ሁለትየመረጃሠብ ሳቢዎች (በሙያየላብራቶ **ባለሙቭ**) እንዲሁምሁለትአማካሪዎችይሣተፋሉ፡፡

የጥናቱማብራሪያ፡ ጥናቱትኩረትሚያደርገው ባወባ በሽታአም ባሆኣው ባቫቭቫክስ ባዛ ራሳቭፔ ባወባ መ ቲፔትት (ክሎሮክሊት) መላመታ ላቭ ኣው፡፡ በሸዋሮቢፔ ጸተማ ጎ፣ ኃቢቨ በሚታጸሙባወባ ታማሚዎኖ ላቭ ቫቭቫክስ ባተባለው በሽተ አም መ ቲቱት (ክሎሮጺት) መላመታ በተመለጸተ ኣው፡፡ በአሁትወቅፔ ክሎሮጺት ለዚህ በሽታ አትተኞ ተረ መ ቲፔ ሆ ዕቨ ለ ለ ኣው፡፡ ትር ት በተለቨብ ባአለም ክሦሎኖ ብሎም በኢፔፑእቨ ቭህ መ ቲፔ ጸበሽታውአም ው ር ዕባተላመተ መሆችት ጎ ኖ ቨሳቨሉ፡፡ በሽተ አም ው ዛ ራሳቭፔ ጸመ ቲቱ ር ጸተላመተ በሽታው ሳቭታት ቀር በሀ ር ላቭ ባተለቨብ ማህበራዊ ኢኮ ሚቨዊ ኖ ሮናት ቨስጸፔላል፡፡ መተ ማሪም ቭህ ባመ ትቲፔመላመታ ወተተለቨብ ባሀ ራቱ ክዎል በመስዌፔ ባባሰ ኖ ር ቭወነ ራል፡፡ በመሆችምቭህት ባመ ቲፔ መላመታ በዚህ አካባቢ ቨለውት ስር ፔ ማወቅ አስወላ ፡ውት ባመጸላጸቨመት ታ ለመቀባስ ዋችኞ ብአፔ ኣው፡፡

የጥናቱቅደምተከተል: በመ መርቨ ሦስፔ ቀ ፔ ዕባተመላለስሽ/ህ መታህቲፔ ፔወስ ለሽ/ህ። በክሂህም ቀ ፔ ባተም ምርመራ ባህክም ክፔፔል ቭተረ ልህል/ሻል በቀኃ ባም በሰባተቾውም ቀት ጸዚቨም በተጸተተቭ ለሦስፔ ሳምትተፔ ለክፔፔል ፔመላለሳለህ/ሽ።

ሥጋትናጉዳት:ለምርምሩ ባተም መ በሚወሰታብፔ ወቅፔ ሊሰማህ/ሽ ጸሚናሳው መነ0 ስሜፔ ው ምትም አቭትፔ ፡ ፔ አቭተርስብህም/ሽም።

ጥቅም:ጥናቱ ላይ በመሳተፍሽ የተለየ ጥቅምአተ ፖም፡ ፡ ትር ት ባመ መርቨውመታህቲፔ ጸልወወሰህ/ ጸልወወሰሽ ሌላ መ ሊፔ ቭሰኀ ህል/ሻል

ሚስጥራዊነት:ለጥናቱ የምትሠጭው ማንኛውም መረጃ በሚስጥር እንደሚያዝ እናረጋግጣለን።

የጥናቱ ተሣታፊ መብቶች:በጥናቱ ላይ የምትሣተፊው ባንቺ ሙሉ ፈቃደኝነት ብቻ እንደሆነ እያሣወቅን በጥናቱ ላይ ያለመሣተፍ ወደ ጥናቱም ከገባሽ በሁዋላም በፈለገሽ ሠዓት አቋርጦ የመውጣትም መብት እንዳለሽ ልንገልፅልሽ እንወዳለን። በጥናቱም ላይ በመሣተፍ ማንም ተፅዕኖ አያሳድርብሽም በጥናቱም ላይ ያለሽን ጥያቄ ሁሉ የመጠየቅና የመረዳት ባለሙሉ መብት ነሽ።

በተጨማሪ መረጃ ማነጋገር ብትፈልጊ ማንኛውም ጥያቄ ቢኖርሽ አሁን ወይም ሌላ ጊዜ የሚከተሉትን ሠዎች በሚከተለው አድራሻ ማግኘት ትችያለሽ።

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አቶእንዳለውዘመት ስልክ. ቁ. 0912071295.

CONSENT FORM

በሽዋሮቢትጤናጣቢያየወባበሽታመድሐኒት(ክሎሮኪዩን)

ፈዋሽነትንበተመለከተበሚደረገውጥናትበምችለውቋንቋማብራሪያተሰጥቶኝበጥናቱእንድሳተፍበተጠየቅኩትመሰረትየጥናቱንዓላ ማናጥቅምበመረዳትያለምንምከፍያአስፈላጊየሆነውንመረጃለመስጠትፍቃደኛመሆኔንበፌርማየአረጋግጣለሁ።

የተሳታፊውፌርማ _____ ቀን _____

ANNEX 2. QUESTIONNAIRE AND DATA SHEETS

QUESTIONNAIRE ON DEMOGRAPHIC AND CLINICAL CONDITION OF THE STUDY PARTICIPANTS (English version).

General instructions- For all the close ended questions please give your responses by circling the number containing your answer(s) and in case of the open ended questions please write your responses on the space provided.

PART ONE: **SOCIODEMOGRAPHIC FACTORS**

1. Code _____

2. Address/Residence / 1. urban_____ **2.** rural_____

3. Age in year _____

4. What is your religion?

1. Orthodox 2. Muslim 3. Protestant

4. Catholic 5. Others specify_____

5. What is your ethnicity?

1. Amhara 2. Argooba 3. Oromo

4. Tigre 5. Gurage 6. Others specify_____

6. What is your marital status?

1. Single 2. Married

3.Widowed 4. Divorced

7. Your educational status?

1. Unable to read and/or write 2. Able only to read and/or write

3. Primary school (1-8) 4. Secondary school (9-12)

5. College and /or university (12+)

8. Your Occupation

1. Government employee 2. Farmer 3.Merchant

4. House wife 5. Daily labour 6. Student

7. NGO employee 8. Unemployed 9. Other (specify) _____

9. Average monthly income of the family (ETB) _____

PART TWO: CLINICAL CHARACTERISTICS AND ATHER IMPORTANT INFORMATIONS

1. Duration of illness before you came the Health Centre _____

2. What symptoms you fill now? _____

3. Did you have a history of fever for previous 48 hours? 1.Yes 2. No

4.Did you take any medication before coming to the Health centre? 1.Yes 2. No

5.If yes, for what purpose did you take? _____

QUESTIONNAIRE ON DEMOGRAPHIC AND CLINICAL CONDITION , OF THE STUDY PARTICIPANTS (Amharic version).

<u>አጠቃላይመመርያ</u>

በመጠይቁላይላሉትየምርጫጥያቄዎችመልስዎትንየያዘውንፊደልበመምረጥይመልሱ::የጽሑፍጥያቄዎችንደግሞለመልስበተሰጠ ውቦታላይይጻፉ::መረጃሰብሳቢዎችምመጻፍናማንበብለማይችሉታሳታፊዎችጥያቄዎችንበማንበብናመልሶቻቸውንበመጻፍሊተ ባበርዋቸውይገባል::

ክፍልአንድማህበራዊ መረጃዎች

1. ስም (ኮድ) _____

2. አድራሻ/መኖሪያ 1. ገጠር 2. ከተማ

3. ዕድሜ _____

4. ሐይማት

1. ኦርቶዶክስ 2. ሙስሊም 3. ፕሮቴስታንት

4. ካቶሊክ 5. ሌላ (ይገለፅ)_____

5. ብሔር

1. አማራ 2. argooba 3. ኦሮሞ

4. ትግሬ 5. ጉራጌ 5. ሌላ (ይገለፅ)_____

6. የትደር ሁኔታ

1. ያላገባች 2. ያባች

3. ባል የሞተባት 4. የተፋታች

7. የትምርት ሁኔታ

1. ማንበብ መፃፍ የማትችል 2. ማንበብና መፃ የምትችል
3. የመጀመሪያ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12)
5. ኮሌጅ/ ዩኒቨርሲቲ (12+)
8. የሥራ ሁኔታ

1. የመንግስት ሠራተኛ 2. ገበሬ 3. ነጋዴ
4. የቤት እመቤት 5. የቀን ሠራተኛ 6. ተማሪ
7. መንግስታዊ ያልሆነ ድርጅት ሠራተኛ 8. ሥራ አጥ 9. ሌላ (ይገለፅ)
9. አማካኝ ወርሀዊ ገቢ _____

ክፍል 2፡ሌሎችጠቃሚመረጃዎች

1.የበሽታውምልክቶችመታየትከጀመሩአንስቶለሕክምናእስከምትመጡያለውጊዜ_____
_____

9.በሽተኞቹበበሽታውምክንያትየሚሰማችሁስሜት_____
_____

10.ወደጤናጣቢያከመምጣታችሁበፊትየወባመድሐኒትወስዳችኋልሀ.አወ

ANNEX 3. STANDARD OPERATING PROCEDURES

1. Blood collection and smear preparation procedure

- ✓ Label pre-cleaned slides (preferably frosted-end) with patient's name (or other identifier), date and time of collection

- ✓ Clean the tip part of ring finger or heel (for infants) with a cotton swab moistened with 70% alcohol.
- ✓ After being dried, prick the clean part of the finger with blood lancet. If blood does not well up, gently squeeze the finger with thumb and index finger.
- ✓ Wipe off the first drop of blood with clean gauze and use the second drop for smear preparation.
- ✓ Place small drop of blood on microscope slide just before the centre of the slide for thin smear and one large drop of blood at about the centre of the second half of the same slide for thick smear.
- ✓ 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. It should extend ½ to 2/3s of the total slide area. 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 (You should be able to read newsprint through it).So that both thin and thick blood smears are prepared on the same microscope slide.
- ✓ Keep the slide on slide tray at horizontal position to be dried with air but it should be free of dust and protected from flies.
- ✓ Fix only the thin smear with concentrated methyl alcohol for 30 seconds.

2. Procedure for Giemsa's staining

- ✓ Prepare 3% of Giemsa working solution in a buffer solution of PH 7.2(e.g. 3ml Giemsa's stock solution and 97ml buffer) and place in staining trough.
- ✓ Place the slides back to back in staining rack with the smears facing outwards and deep in the staining solution. Overflow the staining solution until the microscope slides are covered completely.
- ✓ Stain the slides for 30 minutes
- ✓ Remove the slides, drain the reagent and rinse gently with tap water that kept in a dish.
- ✓ Drain the water and place the slides on drying rack at vertical position.
- ✓ After being dried, examine each slide microscopically using a 100x oil immersion lens and 10x eyepieces to screen parasitemia, to identify the parasite species and to determine density of the parasite.
- ✓ When malaria parasites are detected in a blood film, the parasite density will be determined by counting the number of parasites present per 200 white blood cells in a thick smear and multiplying by 40 to arrive at an approximate parasite count per microlitre of blood. This is based on the assumption that the average WBC count is 8,000/µl blood.

3. Hematocrit determination

Principle:

The hematocrit measures the volume of packed red cells in a given volume of whole blood. This method uses EDTA anticoagulated whole blood or capillary blood obtained by finger stick.

Procedure:

- ✓ Fill two microhematocrit tubes 3/4 full for each. Each test is done in duplicate.
- ✓ Clay the dry end of each tube and put tubes in HCT sheet holes. Label the sheet with patient's name and carefully transport to centrifuge area.

- ✓ Balance tubes in centrifuge, PUT ON LID, and centrifuge at 5000 RPM for 5 minutes. Record the centrifuge # and groove #'s used for each tube on sheet.
- ✓ After centrifugation, use the HCT reader (e.g. card) to set 0 and 100, then read the HCT% at the top of the packed red cells; read to the nearest 0.5%. Record the HCT% obtained for each tube on the lab sheet.
- ✓ Report hematocrit results.

ANNEX 4. 28 DAYS FOLLOW UP CHART FOR EACH STUDY PARTICIPANTS

Code_____ Age _____Sex _____Address _____

App. Time_____ Telephone No._____

date	Follow up day	Slide code	BF		Parasite density	HCT	Axillaries temp	History of fever	Clinical symptom
			Tropho	gamet					
	0								
	1								
	2								
	3								
	7								
	14								
	21								
	28								

ANNEX5. DECLARATION

I, the undersigned, hereby declare that this thesis finding is my original work and has never been presented for any degree in Jimma University or any other institutions of higher learning in Ethiopia. I also declare the duly acknowledgement of all material sources used for this thesis work.

Name of the student: _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____

This thesis has been submitted for approval with my supervision as a University advisor.

1. **Name of advisor:** _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____

2. **Name of advisor**: _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____

Name of examiner: _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____

Name of Department head: _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____