PREVALENCE OF ASYMPTOMATIC MALARIA INFECTION AND ASSOCIATED RISK FACTORS IN MIZAN-AMAN TOWN, ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PARASITOLOGY.

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Abstract

***Background**: Asymptomatic malaria parasitemia remains an effective transmission pool for malaria infection in a community. But it has less attention in malaria controlling and elimination strategies. Therefore, in order to achieve a malaria elimination strategy, it is crucially important to investigate the magnitude of asymptomatic malaria in different settings of these countries. However; there is no enough information on the prevalence of asymptomatic malaria infection and associated risk factors in Bench Sheko Zone, southwest Ethiopia. This study, therefore, aimed to provide information and help for sustainable malaria elimination.*

***Objective**: To determine the prevalence of asymptomatic malaria infection, hemoglobin levels, and associated risk factors in Mizan-Aman town, Ethiopia.*

***Methods**: A community-based cross-sectional study was conducted from February to April 2019, in Mizan-Aman town, southwest Ethiopia. Socio-demographic data were collected using a semi-structured questionnaire. Plasmodium parasite infection was screened by using microscopy and RDT. HemoCue was used to measure the level of hemoglobin. SPSS version 20.0 was applied for description and Logistic regression statistics to assess the association between the asymptomatic malaria infection and risk factors. P-value <0.05 was used as a cutting value of significance.*

***Results**: A total of 353 participants without malaria like symptoms were enrolled in this study. 17 (4.8 %, 95% CI= 2.57, 7.03) of asymptomatic malaria case were revealed. Of this 12(70.58 %, 95% CI= 65.75, 75.25) was due to P.vivax and 5 (29.41 %, 95% CI= 24.74, 34.25) was due to P.falciparum. The presence of mosquito breeding sites [AOR=6.06 (1.76 – 20.82)], utilization of ITN [AOR=3.51(0.97 – 12.68)], and IRS [AOR=3.95 (1.26 – 12.37)] were significantly associated with asymptomatic malaria. n= 15 (4.2 %, 95 % CI [2.11, 6.29]) out of overall anemia was determined; of these 20 %(3/15) of mild anemia were found among asymptomatic malaria cases. Also, there was a significant association between malaria and anemia [OR=5.786 (1.46- 22.85)] in this study.*

***Conclusion and recommendations**: Asymptomatic malaria is an important public health problem in the study area. Low coverage of IRS, ITN, and proximity of stagnant water in residence had an impact on asymptomatic malaria. Further studies are needs on the burden of asymptomatic malaria using the molecular method, and Bench-sheko regional health office better to scale-up of malaria prevention and controlling tools.*

***Key word:** Asymptomatic malaria, level of hemoglobin, risk factors, southwest Ethiopia.*

List of Abbreviations and Acronyms

ACT ----------Artemisinin-based combination therapy

AOR ---------- Adjusted Odd Ratio

CI ---------- Confidence Interval

COR ---------- Crude Odd Ratio

EDTA----------Ethylene diamine tetra acetic acid

ETB ----------Ethiopian birr

HH ----------House hold

HRP2 ----------Histidine-Rich Protein 2

IRS ----------Indoor Residual Spray

LAMP----------Loop-Mediated Isothermal Amplification

LDH ----------Lactose Dehydrogenase

LLIN ----------Long Lasting Insecticide Treated Net

MOH ----------Ministry of Health

PCR- ----------Polymerase Chain Reaction

PI----------Principal Investigator

RDT ----------Rapid Diagnostic Test

SD ----------Standard Deviation

SNNP ---------- South Nation Nationality Peoples

SOP ----------Standard Operating Procedures

SPSS ----------Statistical Package Social Sciences

WBC ----------White Blood Cell

WHO ----------World Health Organization

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CHAPTER ONE

1. Introduction

1.1. Background

Malaria is a disease, which is caused by protozoan parasites belonging to the genus of *plasmodium*. The parasite requires two hosts for completing their life cycles: the vertebrate and invertebrate hosts(1). The parasites are transmitted by Anopheles mosquitoes (2). Infection initiated after sporozoites are released into the host dermis from infected female anopheles mosquito during a blood meal. Sporozoites enter the bloodstream through penetration of the blood vessels and then invade the liver, which is known" traversal process". After the infection is established sporozoites transforms to the liver stage (exo-erythrocyte) over a subsequent 2-10 days, and then developed merosomes burst and release 40,000 merozoites per hepatocyte into the bloodstream. Merozoite invades erythrocytes and asexual replication occurs in erythrocyte then releasing merozoite infects erythrocyte. The majority of merozoites are passes through the developmental stage of ring, trophozoites and then mature schizonts, respectively. Schizonts released merozoites reinvade new erythrocytes; this stage is responsible for clinical manifestatio ns. A small fraction of merozoites is also differentiated to sexual stage (Gametocytes). This was again taken by a feeding mosquito into the gut where they mature to form male and female gametes. The fertilized zygote develops to an ookinete and an oocyst and finally sporozoites that migrate to the salivary gland (3,4)(Figure 1).

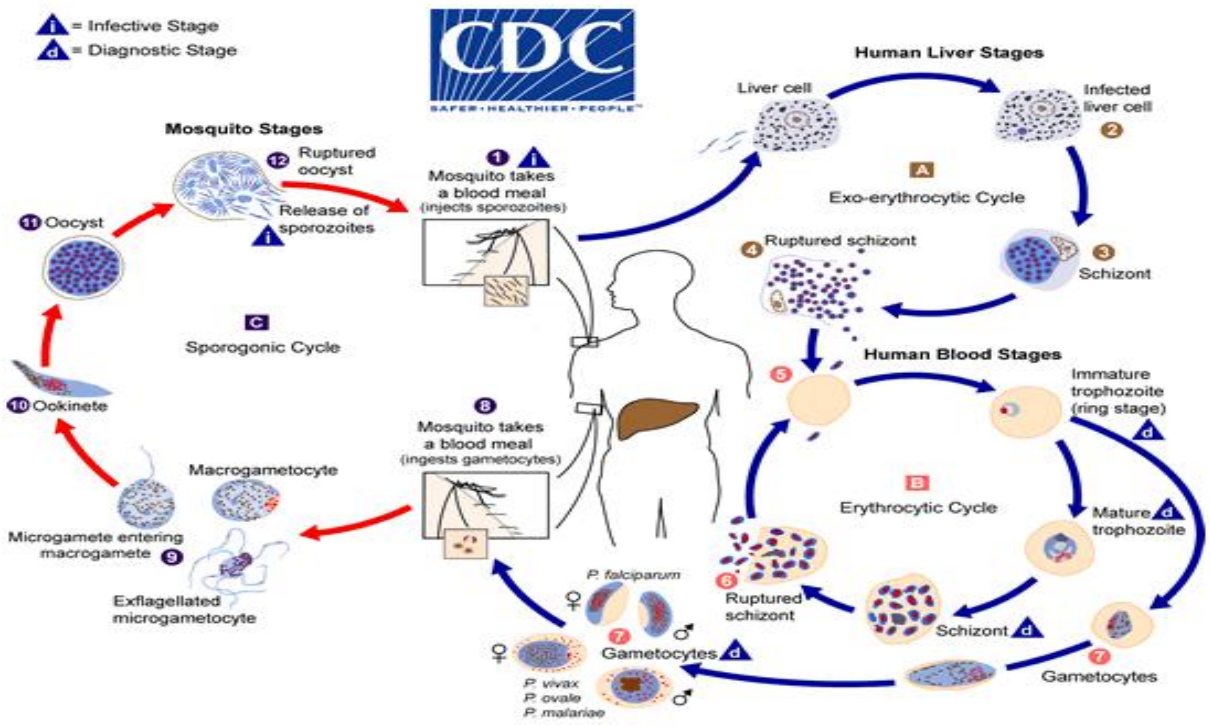


Figure 1. Life cycle of malaria source: https://www.cdc.gov/dpdx/malaria/index.html

Plasmodium specious has a complex life cycle that involves transmission between a mosquito vector and a human host, as well as sexual and asexual developmental stages. In every life cycle stages several hundred parasite proteins are involved for invasion of erythrocyte and intracellular proliferation. Moreover, while the parasite entry to erythrocyte shed many antigens and these parasites encoded erythrocyte-adhesive proteins are also present at high levels in plasma. This may adhere to uninfected erythrocytes and could leading to more clearance from circulation by spleen through the mediate of immunoglobulin G or complement binding to erythrocytes i.e. enhance destruction of uninfected erythrocytes, these are a prominent and perplexing feature of malarial anemia (5).

While rupturing of schizonts stage of parasite it releases merozoite and parasite byproduct i.e. hemozoin, glycosylphosphatidylinositol and other toxic factors in to bloodstream. These byproducts are triggers cytokine cascade production and pyrogenic inflammatory mediators by innate immune cells that in turn stimulate thermoregulatory regions of the brain to increase body temperature, as result of this infected individual develop the symptoms of malaria (6). However,

there is a clinical manifestation differences between the malaria infected individuals. These differences are poorly understood between the symptomatic and asymptomatic individuals. But, the general state of health and physiological conditions of host immunity variations, host genetic predisposition and parasite factors are involve in the virulence of the asymptomatic malaria infection (7).

The releasing cytokines have very important role in hemoglobin level; this cytokines are triggering the inflammatory response of host immunity and increase the destruction of erythrocyte. Therefore, severity of anemia is depends on the parasite density. In asymptomatic malaria infected individual released cytokines might be inhibited the work of Th_1 cell and macrophage this may be decrease erythrocyte destruction (8). In high malaria transmission settings infected adolescent and adulthood are more likely to be asymptomatic due to disease controlling immunity development through repeated infection occurrence. As result of this anemia prevalence is decrease; where as in children and pregnant women are more like to be anemic. But in lower transmission settings anemia may occur at all ages, although the children and pregnant women who are more likely to be anemic (9).

Asymptomatic malaria-infected individuals are harboring blood-stage malaria parasite and they have not been experienced clinical manifestation. Hence, they are not seeking treatment. In most malaria-endemic settings, asymptomatic infections outnumber symptomatic infections (10). In seasonal transmission area can also persistently occur in dry season, these suggest that occurrence of outbreak malaria after annual rain (11). Therefore, success of malaria elimination strategies relies on the ability to find and treat the asymptomatic reservoir (10). Asymptomatic reservoir is might be a composed of submicroscopic and microscopic parasitemia. In both high and low transmission settings (12). Asymptomatic infections are often undetected and untreated; this will become a major source of gametocytes for local mosquito vectors. Then, the gametocyte could be infectious to mosquitos. This will increases the morbidity and mortality of malaria in seasonal malaria transmission area of Africa (13,14).

Across sub-Saharan Africa where the disease is holoendemic, most people are infected by *P.falciparum*, and the majority of infected adults rarely experience overt disease. This could be a result of frequently exposing to malaria infection could develop acquired immunity against

malaria. This acquired (adaptive) immunity helps to regulate the release malaria associated host cells damaging toxins or cytokines. Therefore, these individuals may not develop clinical disease and this affect the extent of malaria morbidity with a given parasite setting. Moreover, the acquired immunity would conferring protection against parasitemia, which affects the density of parasites and premunition (providing protection against new infections by maintaining a low-grade and generally asymptomatic parasitemia) (15).

In Ethiopia, the transmission of malaria is seasonal in many setting of the country with nearly perennial transmission in some areas. However, the transmission is influenced by many factors including climates (temperature, rainfall and relative humidity), topography (altitude, surface hydrology, land vegetation cover and land use), and human settlement and population movement patterns. Malaria occurs at up to the 2000 meter elevation; but occasionally occurs above 2000 meter elevation and peak transmission occurs from September to December, following the main rainy and minor malaria transmission period from April to June, following a short rainy season(16,17). The epidemiology of malaria in Ethiopia was decreasing from 4.5% in 2011 to 1.2% in 2017. However, in 2019 malaria operational plan report the burden of malaria is more than 80% among adults and children (18).

Accurate diagnosis of malaria is very crucial for controlling of local malaria transmission and towards the long-term goal of elimination. Currently there are many diagnostic techniques for malaria including microscopic diagnosis test, quality-assured conventional rapid diagnostic test (RDT) and molecular diagnostic techniques. However, most of these techniques have low sensitivity toward low density parasitemia (<100 parasites/μl), a common characteristic of asymptomatic malaria (19). In contrast nucleic acid amplification based diagnostic techniques such as polymerase chain reaction (PCR) and Loop-mediated isothermal amplification (LAMP) diagnosis techniques are highly sensitive, can detect low-density (5-10 parasites/μl) of asymptomatic malaria infections (20).

Sub-microscopic asymptomatic infections appears to be higher in low transmission settings than in high transmission settings (21). Therefore, in order to reduce the rate of asymptomatic reservoirs it is important to investigate the burden of asymptomatic malaria in these moderate to high transmission area using the most available and affordable techniques such as blood film examination and malaria rapid diagnostic test.

1.2. Statement of the problem

Globally, the WHO malaria report estimated 228 million malaria cases in 2019, with 9,000,000 more cases from the previous year. Among this WHO Africa region bears 213,000,000 cases. In contrast, death due to malaria was decreased from the previous year, an estimated from 435,000 to 405,000 of death (22). In the Ethiopian context, there was a decline in malaria death in 2015 (23). However, there was an increase in malaria transmission rate in 2016 as compared to previous years (16). Higher incidence of malaria contributes to school absenteeism on children, child labor when a parent is sick (24), low productivity due to illness, permanent neurological, and other damages were caused on infants (25). Moreover, it has impacts on anemia, cognitive impairment, intrauterine growing retardation, and prematurity in pregnant women as a result of both severe and uncomplicated episodes of malaria infection(9,26).

Ethiopian national malaria control strategy planned to achieve and sustain zero indigenous malaria transmission by 2020 in selected 239 low transmission districts and eliminating from Ethiopia by 2030. It has been suggested that this strategy could be attained through the Scale up of vector control intervention, early diagnosis and prompts treatment using microscopy and RDT (18). The ministry of health (MOH) implemented different measures that can reduce the burden of malaria. The mass distribution of insecticide-treated bed nets (ITNs), together with increased utilization of long-lasting ITNs (LLINs), indoor residual spraying (IRS) (27) and adoption of Artemisinin-based combination therapy (ACT) resulted in substantial declines in malaria-related morbidity and mortality in Ethiopia (28).

However, none regular use of LLINs (29), coverage gap in vector controlling tools, change the behavioral pattern of mosquito (Exophagy), the resistance of LLINs and IRS (30), and the hypnozoite reservoir of infection in endemic communities (31) are big challenges for elimination of malaria. Moreover, the presence of asymptomatic malaria cases in a community is a major challenge to achieve malaria elimination strategy as the reservoir gametocyte stages of the parasites. These are the infective stages to the vectors. As a result, these asymptomatic cases are contributing to continuous malaria transmission in the community (32).

For instance, the prevalence of asymptomatic malaria in western Cambodia, China–Myanmar border, and India was 9.1% (33), 23.3% (34), and 20.7% (35), respectively. Also, a review of asymptomatic malaria in African countries indicates asymptomatic cases were still a problem (36). Hence, the prevalence in Gabone 18.8% (37) and in Nigeria 69.9 %(38). Furthermore, In Ethiopia the prevalence was ranging from 0.93% to 21.5% in different settings; 0.93% in Butajira (39), 8.2 % in South-central Oromia (40), 21.5% in Gambella region (41) and 4.1% in Arba Minch town (42).

Therefore, to address the 2030 zero malaria transmission goals, effort should be made to investigate the burden of asymptomatic malaria cases in both high and moderate transmission settings of the country. Mizan-Aman town is located at a moderate malaria transmission area and there is a lack of study on the burden of asymptomatic malaria and associated risk factors to our knowledge. Therefore, the aim of the study is to determine the prevalence of asymptomatic malaria, the level of hemoglobin, and associated risk factors in the Mizan-Aman town community, southwest, Ethiopia.

1.3. Significance of the study

Knowing the prevalence of asymptomatic malaria infection and associated risk factors among the community of moderate and high transmission areas is very crucial for informing policymakers to design effective strategies for achieving long term goals of malaria elimination. The result of the current study results will be used by the local malaria control office, MOH, and other stakeholders to plan effective malaria prevention and control strategies. In addition, it will help to evaluate the effectiveness of malaria interventions being implemented in the study area. Based on the findings, the management of asymptomatic carriers could also help to reduce the risk of malaria transmission in the communities. Furthermore, this study will be used as a baseline for further work on asymptomatic malaria in the area.

CHAPTER TWO

2. Literature review

2.1. Asymptomatic malaria infection definition

The clinical manifestation of malaria infection is different among individuals. Immunity of the individuals is a determinant of clinical manifestation. Different studies define the asymptomatic malaria infection is the detection of blood stage parasite with any density of parasitemia in the absence of malaria related symptoms in a specific time frame. This can be persist for several months to years without showing the clinical manifestations, this may due to permutation (43). In perennial malaria transmission area presence of asymptomatic malaria, parasite carriers were responsible for persistent malaria transmission (44). Therefor for achieving a malaria elimination program there must be measuring the impact of malaria control interventions with parasite prevalence is critical (12). Many studies have tried to characterize the asymptomatic malaria infections in a great variation between studies through the use of conventional malaria diagnosis techniques.

2.2. Studies on asymptomatic malaria prevalence and plasmodium specious distribution

According to cross-sectional surveys conducted in malaria-endemic areas along the Thailand–Myanmar border, in western Cambodia, and south-western Vietnam; the prevalence of asymptomatic malaria was 5 % (229/5111) (45). Another study conducted across Haiti; among the reproductive women age group, 3.0 % (16/563) of participants were positive for asymptomatic malaria (46). A relevant study conducted in Maynas province, Northern Peruvian Amazon; among the asymptomatic population malaria has revealed 4.9% (57/1167). The *Plasmodium* species proportion of this study was 10.5% (6/57) due to *P. falciparum*, whereas *P. vivax* accounted for the majority of infections (47). The survey conducted in Kayah State, eastern Myanmar; was also revealed 1.44% (7/485) of *Plasmodium* infection among asymptomatic participants. Of this infection five were positive for *P. falciparum*, one for *P. vivax* and one for mixed infections (*P. falciparum* and *P. vivax*) (48).

Different studies conducted in African countries showed the presence of asymptomatic malaria infection. According to a community-based screening survey in Zanzibar, the prevalence of asymptomatic malaria in that low transmission area was 1.0 % (10/997) through the RDT (49). Another household cross-sectional survey carried out in Korhogo and Kaedi, West Africa 12.4% (823/6693) and 0.2 %(18/9165) of parasitemia was determined through microscopy, respectively; whereas through the use of RDT 10.5% (703/6693) of infection was detected in Korhogo village (50). In the low transmission setting of the African counter of the Zambezi region, Namibia was identified 0.8% (16/1919) of *P. falciparum* malaria infection was identified among the sub-patient participants. In this study, only *P. falciparum* species were identified (51). The household survey was conducted in Abuja Municipal Area, Nigeria 421/602 (69.9%) of asymptomatic malaria have reveled among households linked to malaria patients attending the health facilities (38), another epidemiological survey was conducted in the endemic area of Southwest Nigeria, showed 34.6 % (117/338) of asymptomatic malaria infection (52). In Gabon, central Africa also 18.8% (85/451) of asymptomatic malaria was determined through cross-sectional study; only *P. falciparum* was detected (37).

In Ethiopia national malaria report, *P. falciparum* and *P. vivax* accounted *60%* and 40% of cases, respectably (16). Some studies conducted in different part of Ethiopia, have evidences on the presence of asymptomatic malaria in community level. In the residents of Dembia district, North Gondar zone conducted cross-sectional study among adults age 6.7 % (56/832) of asymptomatic malaria was determined through RDT. Of this due to *P. falciparum* 46(82%), due to *P. vivax* 5(9%) and due to mixed species (Pf + Pv) 5(9%) (53). Other study conducted in Debre Elias district, East Gojjam zone; the prevalence of asymptomatic malaria among healthy participants was 4.8% (16/333) (54), also in West Armachiho district, among asymptomatic migrant laborers 18.4% (71/385) of malaria was revealed. Of this due to *P. falciparum* 70.4%, due to *P. vivax* 9.9%, and due to mixed infections 19.7% (55). Among schools children in Oromia regional state 0.56 % (117/20,899) of asymptomatic malaria was determined (56). Other study conducted in West Arsi Zone, Oromia Region; malaria prevalence was 8.2 % (90/1,094). Out of positive subjects 66.7 %, 25.6 % and 7.7 % were *P. falciparum*, *P. vivax* and mixed infections, respectively (40); In Jimma town local community conducted survey, among malaria suspected cases 29.8 % (428/1434) of malaria was confirmed. Of this revealed *Plasmodium* species was

76.4 %, 22.7 %, and 0.9 % due to *P. vivax, P. falciparum* and mixed infection, respectivly (57). In southern, Ethiopia conducted study in the pastoralist community of Benna Tsemay district was also revealed 6.1 % of malaria. Of this *P.falciparum*, *P. vivax* and mixed infection was 64.3 %, 21.4 % and 14.3 %, respectively (58). In Arba Minch Town, conducted study was revealed 9.1% (31/341) of asymptomatic malaria among pregnant women. The plasmodium species distribution of this study was 38.71%, 48.38%, and 12.9% of *P. falciparum*, *P. vivax* and mixed infection, respectively (59).

2.3. Studies on asymptomatic malaria and associated risk factors

A different study conducted in sub-Saharan African country's showed a number of risk factors for asymptomatic malaria infection. According to a study conducted in Gabon, central Africa identified Age, education, suburban area, and water body near home have been identified as a risk factor for asymptomatic malaria infection (37); Similarly in Nigeria, bush around homes was found significantly associated with asymptomatic malaria infection (38). Studies conducted in Ethiopia, also showed education level, migration, number of visits, outdoor sleeping, and bed net utilization were identified as a risk factor for asymptomatic malaria infection in west Armachiho district, Northwest (55), similarly in Dembia district, northwest Ethiopia, conducted study sex, age, utilization of ITN, and stagnant water were identified a significant association with asymptomatic malaria infection (53); likewise, in Debre Elias district, East Gojjam zone conducted study occasional utilization of ITN, not using ITN, house with eave, previous history of malaria and family history of malaria infection were significantly associated with malaria infection (54). Similarly, conducted study in Oromia region reveled IRS, LLIN (56), Being males and younger age was significantly associated with malaria (40). A study conducted in Southern, Ethiopia revealed pregnancy, saving mosquito net for later use (58), and ITN (59) was had a significant relation with asymptomatic malaria.

2.4. Studies on asymptomatic malaria infection and Anemia

Malaria can cause anemia in different mechanism. The occurrences of malaria anemia may be due to the destruction of infected erythrocyte, impaired erythropoiesis and about 90 % of the clearance of uninfected erythrocyte (60) are factors. The clinical consequence of malaria anemia is depends on the transmission intensity. In high transmission settings peoples may receive one

malaria infectious bite in each day, so the adolescent and adulthood may become asymptomatic due to the antdisease immunity development and the prevalence of anemia is decrease. But young children infected with malaria may develop anemia. In low transmission settings in all age group symptomatic malaria may develop and anemia also has occurred (61).

A study conducted in Indonesia showed among asymptomatic individuals 32.8% of anemia was determine; among those 13.1%,15.8% and 3.4 % had mild, moderate and sever, respectively(62). In Nigeria, among asymptomatic infected individuals 55.2% (123/223), was determine 28.5%, 45.5% and 14.6% of mild, moderate and severe anemia (63), respectively. In Arba Minch Town and Benishangul Gumuz Regional State, Ethiopia anemia was reveled 34.6 % (118/341) and 73.76% (194/263), respectively (59,64).

The aims of this study was to determine the magnitude of asymptomatic malaria infection, level of hemoglobin and associated risk factors in Mizan-Aman town, southwest Ethiopia.

CHAPTER THREE

3. Objectives

3.1. General objective

- To determine the prevalence of asymptomatic malaria infection, level of hemoglobin and associated risk factors among asymptomatic individual in Mizan-Aman town, Ethiopia.

3.2. Specific objectives

- To determine prevalence of asymptomatic malaria infection among asymptomatic individual.
- To assess associated risk factors to asymptomatic malaria infection among asymptomatic individual.
- To determine the levels of hemoglobin among asymptomatic individuals.

CHAPTER FOUR

4. Materials and Methods

4.1. Study area and period

The study was conducted in Mizan-Aman town, located in Bench-Sheko Zone, South West Ethiopia, between February 19 and April 19, 2019 G.C. Bench-Sheko Zone is one of zones in Ethiopian Southern Nations, Nationalities, and Peoples Region (SNNPR). The altitude of the town is 1,451 meters above sea level. The average annual temperature and rain fall ranges from 15 ^{0}c to 27^{0}c and 400-2,000 mm, respectively. Coffee planter is the primary source of income. The capital of the zone is located at a distance of 574 km south west of Addis Ababa, capital city of Ethiopia. According to the national malaria report the current study setting was moderate malaria transmission area (16). Five health posts, one health center and one teaching hospital are found in study setting. Four primary, one secondary and one secondary and preparatory government schools, and also one primary and secondary private school are found in the town.

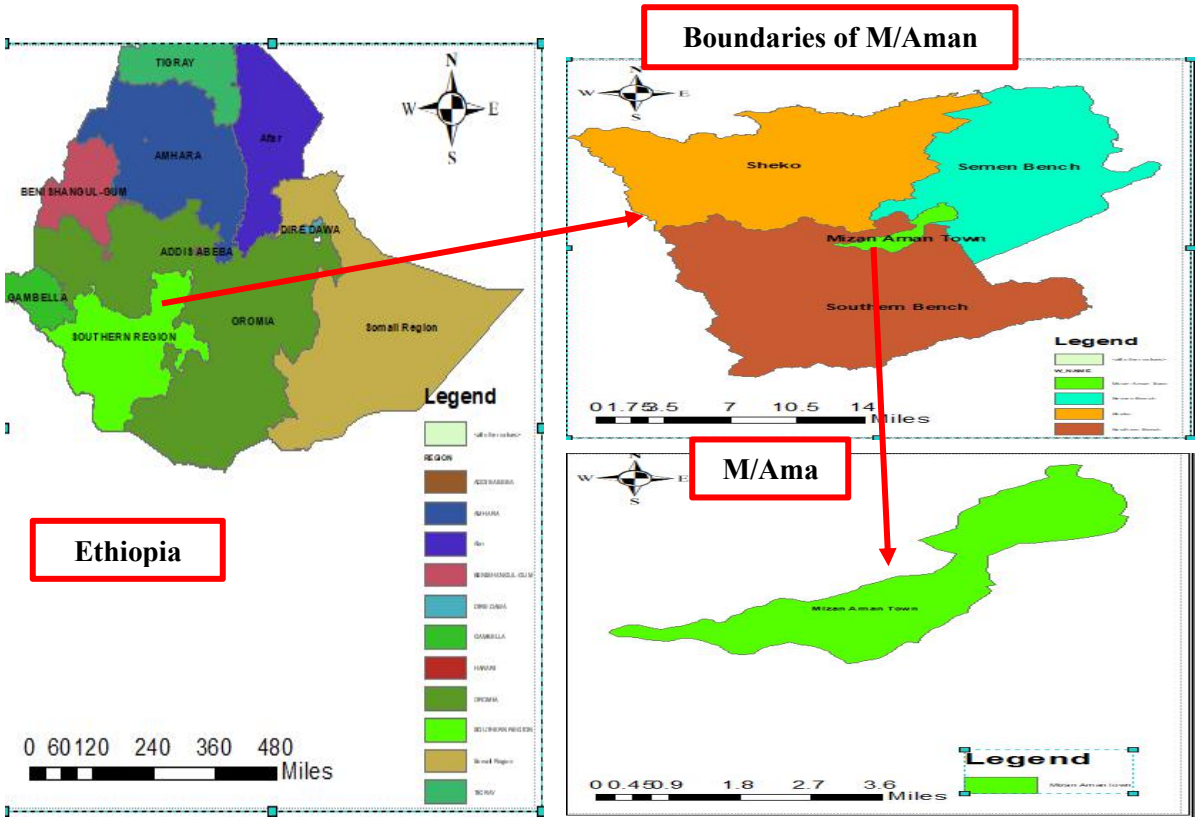


Figure 2. Map of study area

4.2.Study design

Community based cross-sectional study was conducted in selected kebeles of Mizan-Aman town, Southwest Ethiopia.

4.3.Population

4.3.1. Source population

The source population was population residing in the Mizan Aman town at least for the last one year.

Study population

All individuals who fulfill the inclusion criteria and giving consent in selected kebeles.

4.4.Sample size and sampling technique

4.4.1. Sample size determination

The required sample size was determined by using a formula of single population proportion.

$$n = \frac{(Z\alpha/_2)^2 \times p(1-p)}{d^2} \times DE , \quad \frac{(1.96)^2 \times 0.067\ (1-0.067)}{(0.0335)^2} \times 1.5 = 321$$

Where*:*

n = Sample size

P = Expected proportion of prevalence of malaria is 6.7 % (53).

Z $\alpha /_2$ = 1.96 (at 95% confidence level)

d = 0.0335 % marginal error, if P is below 0.1 (10%) and above 0.9 (90%), d as a half of P to obtain a large sample size (65)

DE = Design effect is 1.5

Based on the above assumptions the minimum sample size was 321 required. To minimize errors arising from the probable occurrence of non-compliance (non-respondent rate), 10% of the sample size was added and finally 353 study subjects were included in the study.

4.4.2. Sampling techniques

A multi-stage sampling technique was used to select the study unit. The town of Mizan-Aman has five kebeles; of those two of them (shesheka and kometa) were selected randomly for this study. Total populations of shesheka and kometa kebeles are 8,455 and 7,801 and, households in each kebeles are 1,726 and 1,592, respectively. The calculated sample size 353 was divided for SNNP average family size i.e. (4.9), so that 72 households (HH) were estimated to include in the current study, however 97 HH were included. In each kebeles 51 and 46 households were selected randomly from shesheka and kometa through random table, respectively. All available individual family members in the selected households at the time of data collection were included until the sample size reaches. Venous blood was collected for the determination of asymptomatic malaria and hemoglobin level; and semi-structured questioners were also used to assess socio-demographic, socio-economic characteristics and associated risk factors for asymptomatic malaria infection from February 19 to April 19, 2019 G.C. Written consent was obtained from participants and their parents/guardians for under 5 children.

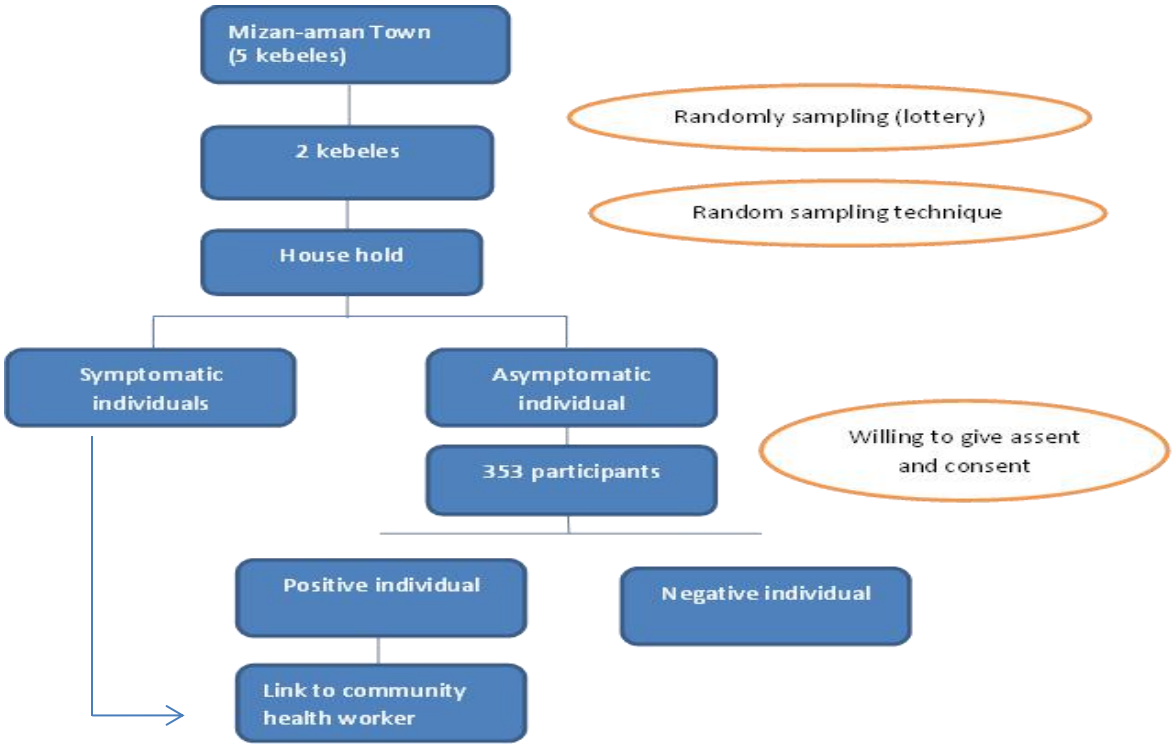


Figure 3. Schematic presentation of sampling procedure in mizan aman twon, 2019.

4.5.Study variables

4.5.1. Dependent variable

Asymptomatic malaria infection

Hemoglobin level

4.5.2. Independent variables

Age in year

Sex

Educational status

Place of residence (locality)

Occupational status

Monthly estimated income

Utilization of ITN

Indoor residual spray coverage

Replant utilization

Presence of parasites in blood

Resting behavior

Previous history of fever

Previous self-medication history

Previous malaria history

Presence of breeding site around residence

House wall type

House floor type

Latrine type

4.6.Eligibility criteria

4.6.1. Inclusion criteria

- All individuals above two years of age, auxiliary body temperature < 37.5 ^{0}C, individuals consented for the study and permanently residing for at least one year in study setting were included in the current study.

4.6.2. Exclusion criteria

- Those individuals who took antimalarial treatment within two weeks at the time of data collection, having malaria like symptom and residing less than one year in a community were excluded from this study.

4.7. Data collection

4.7.1. Questionnaire

Socio-demographic, socio-economic characteristics and associated risk factors data were collected using semi-structured questionnaires (Annex-I).

4.7.2. Specimen collection and processing

4.7.2.1 Venous Blood Sample Collection

Before commencing blood sample collection enough information was given to participants about the study; then for individuals wiling and decide to participate in the study auxiliary body temperatures was measure with digital thermometer by community health workers; and from participants with <37.5 ^{0}C body temperature and did not took antimalarial treatment within the previous two weeks approximately 3-4 ml of venous blood sample was drawn with EDTA tube by experienced laboratory personnel with aseptic technique(Annex-II).

4.7.2.2 RDT Testing

RDT (*care start* ^{TM} Malaria HRP2/pLDH (Pf/PAN) Combo) was used on spot to detect Plasmodium species. It works through the lateral flow; by using mobile phase monoclonal antibodies prepared against target malaria antigens conjugated with the gold particles; and the test area contains immobilized monoclonal antibody which catcher the antibody antigen complex give color. The RDT histidine-rich protein 2 (HRP2) used to detect *P. falciparum* and lactose dehydrogenase (LDH) for *Plasmodium vivax*.

4.7.2.3 Hemoglobin Determination

The EDTA tube blood samples were taken to Mizan-tepi university teaching hospital laboratory, then Hemoglobin level was determined with HemoCue® Hb 301 System following the manufacturer's instruction. Measure the absorbance of whole blood at an Hb/HbO2 isobestic point; dual wavelengths (506 nm and 880 nm) for Hb measurement and turbidity compensation. Quality control built in"selftest", optional liquid controls.

4.7.2.4 Microscopic Examination

Dried blood spot (DBS) was also prepared for each blood sample with whatman™ filter paper for molecular diagnosis. Thick and thin blood smears were also prepared, stained with 10 % Giemsa stain for 10 minutes. Giemsa stain contain methylene blue it stain the parasite cytoplasm and eosin stain is acidic stained parasite chromatid give rad/pink color and examined for the presence of malaria parasites by laboratory technologist and parasite density was calculated by counting the number of asexual parasites per 200 leukocytes in the thick blood film; while considering a slide as negative after counted 500 leukocytes. When thick films were positive, thin films were read for species differentiation. 8,000/µl white blood cell is assumed normal reference range. The Parasite density per µl was calculated using the following formula:

$$\text{Parasites / µL blood} = \frac{\textbf{Number of parasite counted} \times \textbf{8,000 WBC/ µL blood}}{\textbf{Number of WBC counte}}$$

4.8.Data quality assurance

Pre-analytical phase: pre-test was performed for semi-structured questionnaire, data collectors were trained and quality controls were performed for each test procedure before commencing the test.

Analytical phase: the manufacturer instruction and standard operating procedures (SOPs) were strictly followed when running each test.

Post-analytical phase: 10 % of negative and all positive blood film slides were re-examined by blinded independently senior medical laboratory technologist and completeness of data was also checked regularly.

Data analysis

Data entry, data cleaning and coding was performed by using Epi data manager version 4.4.2.1 and then exported to statistical Package Social Sciences (SPSS) version 20.0 software package for analysis. Bivariate and multivariate logistic regression models were used to assess the predictors that contribute for the asymptomatic malaria infection. The results were then summarized and presented by tables, figures and text.

4.9.Ethical considerations

The protocol was reviewed and approved by the institutional review board (IRB) of institute of Health, Jimma University and supportive letter also obtained from School of Medical Laboratory Science. The aim and procedure of the study was introduced to the Mizan-Aman health office and each selected kebele administration before commencing the study. After introducing the aim of study, participants and parents /legal guardians for under 5 children who were volunteer to participate in the study gave written informed consent. Those participants who were positive for malaria and with low level of hemoglobin were linked to the community health worker for treatment and management according to national malaria treatment guide line.

4.10. Plan for dissemination

The result of this study will be submitted to Jimma university school of medical laboratory, institute of health science post graduate study, postgraduate library and bench-sheko zone health office. Effort will be made to present in scientific conference and for publication on peer reviewed scientific journal by incorporating the molecular technic result.

CHAPTER FIVE

5. Results

5.1. Characteristics of the study participants

A total of 353 individuals without malaria like symptoms were participated in the current study, of which 53% (n=187) of them were from shesheka and the rests 47.0% (n=166) were from kometa kebeles. A proportion of sex was approximately equal females in 53.5 % (n=189) and males in 46.5% (164). The mean age of participants was 26 years (±11 SD). The majority of the participants' age was fall in 16-25 age group in 40.2% (n=142). Primary school and student are the predominant educational and occupational status of the participants with 46.5 % (n=164) and student in 32.6% (n=115), respectively. Majorities of the participants estimated monthly income was below 650.00 ETB which was 32.3 %(n=114). More than 90% of the participants were none utilizer of ITN, replants, had a history of fever and history of malaria. More than half of participants were live in IRS sprayed home and had history of self-medication; which was 66.0% (n=233) and 64.3% (n=227), respectively (Table 1).

Table 1. Characteristics of study participants in Mizan-Aman town; Ethiopia, February to April 2019

Characteristics	Frequency n (%)	Characteristics	Frequency n (%)
Address			
Shesheka	187(53.0%)	**Utilization of ITN**	
Kometa	166 (47.0%)	Yes	35 (9.91%)
Gender		No	318 (90.08%)
Male	164(46.5%)	**Use of replants**	
Female	189(53.5%)	Yes	11(3.1%)
Age in year		No	342(96.9%)
≤5	3(0.8%)	**Indoor residual spray coverage**	
6-15	52(14.7%)	Yes	233(66.0%)
16-25	142(40.2%)	No	120(34.0%)
26-35	88(24.9%)	**Previous history of fever**	
≥36	68(19.3%)	Yes	334(94.6)
		No	19(5.4)

Characteristics	Frequency n (%)	Characteristics	Frequency n (%)
Education Status		**Previous malaria history**	
Illiterate	74(21.0%)	Yes	325(92.0%)
Only able to write and read	32(9.1%)	No	28(8.0 %)
Primary school (1-8)	164(46.5%)	**Presence of breeding site**	
Secondary (9-12)	53(15.0%)	Yes	133(37.7%)
College/above	30(8.5%)	No	220(62.3%)
Occupation status		**House wall type**	
Government	40(11.3%)	Wood	143(40.5%)
Farmer	78(22.1%)	Brick	13(3.7%)
House wife	84(23.8%)	Other	197(55.8%)
Merchant	23(6.5%)	**House floor**	
Daily laborer	9(2.5%)	Wood	2(0.6%)
House servant	4(1.1%)	Cement	180(51.0%)
Student	115(32.6%)	Mud	171(48.4%)
Estimated monthly income (ETB)		**Resting behavior**	
<650	114(32.3%)	Indoor	353(100%)
650-1300	37(10.5%)	Outdoor	0
>1300	37(10.5%)		
Self-medication history			
Yes	227(64.3%)		
No	126(35.7%)		

5.2. Microscopy and RDT confirmation of asymptomatic malaria infection

Among 353 asymptomatic individuals n = 8 (2.3%, 95% CI [0.74, 3.86]) of asymptomatic malaria infection was determine and differentiate specious through microscopy examination. Of this 6/8 (75.0 %, 95% CI [70.49, 79.51]) were *P.vivax*, 2/8 (25 %, 95% CI [24.44, 25.55]) were *P. falciparum*. Six subjects were with the parasitemia < 500 parasite per µl of blood, one subject with the parasitemia 920 parasite per µl of blood, and one was in parasitemia 1080 parasite per µl of blood. Whereas the RDT was detected n = 17 (4.8 %, 95% CI [2.57, 7.03]) of asymptomatic malaria infected participants; of this 12(70.5 %, 95% CI [65.75, 75.25]) was due to *P.vivax* and 5 (29.41%, 95% CI [24.74, 34.25].) was due to *P.falciparum*. All microscopically detected malaria was confirmed through RDT, therefore the overall prevalence of asymptomatic

malaria in this study was 4.8% (n=17). No mixed infection was reveled in both of diagnostic technique (Table 2).

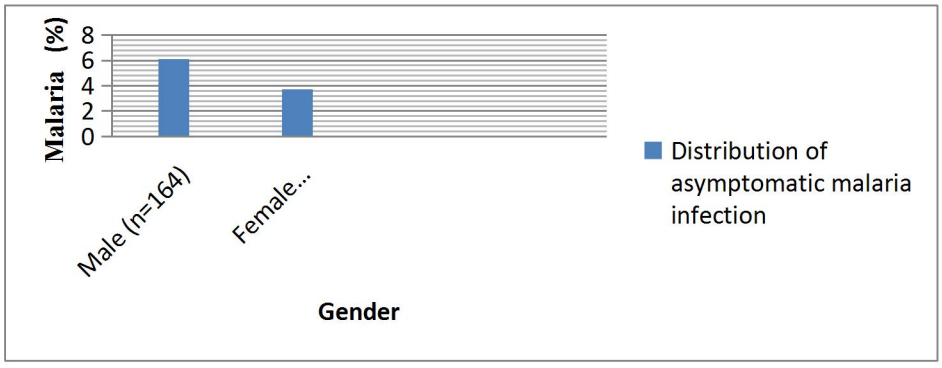
Table 2. Microscopy and RDT confirmed asymptomatic malaria infection in Mizan-Aman town; Ethiopia, February to April 2019

		Microscopy results			
		P. falciparum	***P. vivax***	***Negative***	***Total***
RDT results	***P. falciparum***	**2***	**0**	**3**	**5**
	P. vivax	**0**	**6***	**6**	**12**
	Negative	**0**	**0**	**336**	**336**
	Total	**2**	**6**	**345**	**353**

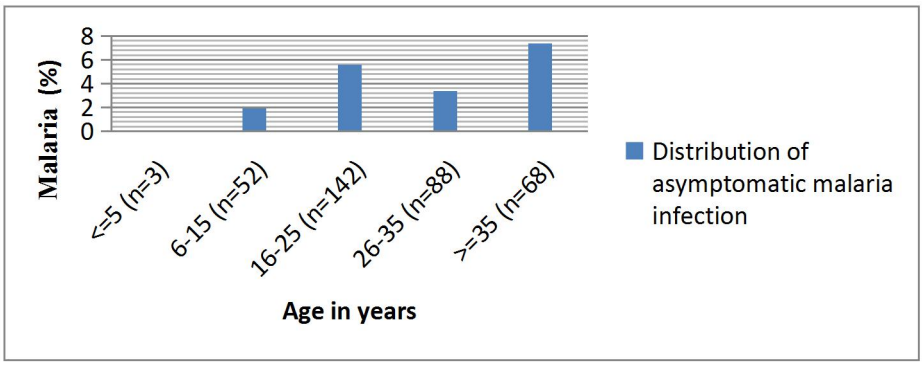
* Number of detected malaria case by both microscopy and RDT

5.3. Distribution of asymptomatic malaria by age, sex and address/kebeles

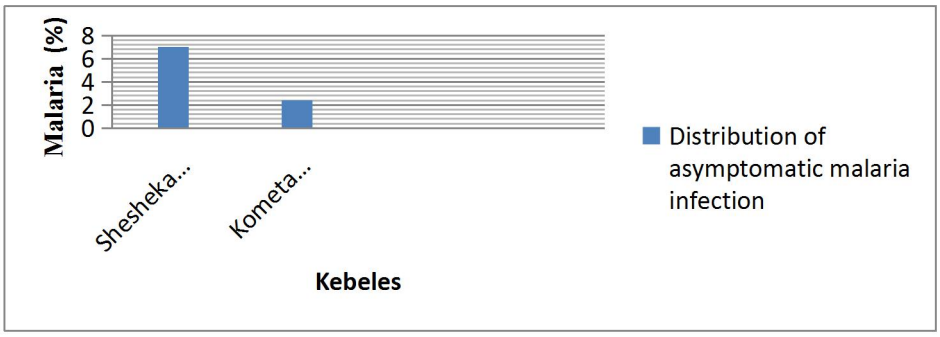
The distribution of asymptomatic malaria among in a specific age groups 7.4 % (5/68) of asymptomatic malaria was observed in ≥ 35 age group and this is the highest prevalence among age group. In male sex group 6.1 % (10/164) of asymptomatic malaria was observed, whereas female sex was 3.7 % (7/189) of asymptomatic malaria was detected. The distribution of asymptomatic malaria between kebeles was different. 7% (13/187) of malaria was detected in shesheka kebele and 2.4 % (4/166) in kometa kebele (Figure 4).



A



B



C

Figure 4. Chart shows the distribution of asymptomatic malaria Vs. gender (fig A), age (fig B) and kebeles (fig C) in mizan-aman town; Ethiopia, February to April 2019

5.4. Risk factors associated with asymptomatic malaria infection

For logistics regression only 13 variables (address, gender, age, educational status, occupational status, estimated monthly income, history of malaria, self-medication, use of antimalarial spray, house wall type, presence of mosquito breeding site, use of replants and utilization of ITN) was fitted. *P-value < 0.25* was used as a cutting value in bivariate logistics regression for a variables candidating to multivariate logistics regression to control confounding factors. $P < 0.05$ used as a cutting value in multivariate logistics regression for significant association. Gender, age, educational status, occupational status, estimated monthly income, previous history of malaria, previous self-medication history, IRS coverage, house wall type was didn't show a significant association with the outcome variable (*P > 0.25*) (Table 3).

Whereas, five variables (Address, use of replant, IRS coverage, presence of mosquitos breeding site and utilization of ITN) were candidate to multivariate analysis as a result of *P.value* < 0.25 in bivariate analysis. Among those in multivariate analysis utilization of ITN, coverage of IRS and presences of mosquito breeding site were significantly associated with asymptomatic malaria infection (*P-value* < 0.05) in the current study.

An individual that none utilization of ITN during bedtime has 4.7 times likelihood to be infected with asymptomatic malaria than those utilizer [AOR= 3.51(95% CI= 0.973 – 12.681)]. Individuals that live in none sprayed with antimalarial home (IRS) has 4 times likelihood to be infected with asymptomatic malaria as compared to those lived in sprayed home [AOR=3.95, (95%CI =1.26 – 12.37)], and presence of mosquito breeding site in living environment has 6 times probability to be infected as compared to those who live in absence of mosquito breeding site in surrounding environment [AOR= 6.06 (95% CI=1.76 – 20.82)] (Table 3).

Table 3. Bivariate and multivariate analysis of risk factors association with asymptomatic malaria infection in Mizan-Aman town; Ethiopia, February to April 2019

Variables		No of infection/Total examined	COR (95 %CI)	*P.value*	AOR (95% CI)	*P.value*
Address						
	Shesheka	13/187 (7%)	1.00		-	
	Kometa	4/166 (2.4%)	0.33(0.106 – 1.034)	0.057*	0.324(03097 – 1.084)	0.67
Gender						
	Male	10/164(6.1%)	1.00	0.3	1	
	Female	7/189(3.7%)	0.592(0.22 – 1.593)			
Age						
	<5	0/3(0%)	0.00	0.999		
	6-15	1/52(1.9%)	0.247(0.028 – 2.182)	0.208		
	16-25	8/142(5.6%)	0.752(0.237 – 2..392)	0.630		
	26-35	3/88(3.4%)	0.44(0.102 – 1.93)	0.279		
	>36	5/68(7.4%)	1		1	
Educational status						
	Illiterate	0/74(0%)	0			
	Only able to write and read	4/32(12.5%)	9(0.339 -11.817)	0.444		
	Primary school (1-8)	7/164(4.3%)	0.624(0.123 – 3.161)	0.569		
	Secondary (9-12)	4/53(7.5%)	1.143(0.197 – 6.641)	0.882		
	College/above	2/30(6.7%)	1		1	
Occupation						
	Government	2/40(5%)	1		1	
	Farmer	4/78(5.1%)	1.02(0.18 – 5.862)	0.976		
	House wife	2/84(2.4%)	0.463(0.063 – 3.415)	0.450		
	Merchant	1/23(4.3%)	0.864(0.074 – 10.081	0.907		
	Daily laborer	1/9(11.1%)	2.375(0.791 – 29.477)	0.501		
	House servant	1/4(25%)	6.33(0.437 – 91.708)	0.176		
	Student	6/115(5.2%)	1.046(0.202 – 5.404)	0.957		

Variables	No of infection/Total examined	COR (95 %CI)	*P.value*	AOR (95% CI)	*P.value*
Estimated monthly income (ETB)					
<650	6/114(5.3%)	2(0.233 – 17.176)	0.528		
650-1300	3/37(8.1%)	3.17(0.315 – 32.09)	0.327		
> 1300	1/37(2.7%)	1		1	
Previous malaria history					
Yes	15/325(4.6%)	0.629(0.136 – 2.901)	0.552		
No	2/28(7.1%)	1		1	
Previous Self-medication history					
Yes	9/227(4.0%)	0.609(0.229 – 1.620)	0.32		
No	8/126(6.3%)	1		1	
Spread antimalarial spray (IRS)					
Yes	5/233(2.1%)	1		1	
No	12/120(10.0%)	5.06(1.741 – 14.743)	0.003*	3.95(1.261 – 12.374)	0.004
House wall type					
Wood	2/143(%)	1		1	
Brick	0/13(%)	0.00	0.999		
Other	15/197(%)	5.8(1.307 – 25.825)	0.021		
Presence of breeding site					
Yes	13/133(9.8%)	5.85(1.86 – 18.34)	0.002*	6.06(1.765 – 20.826)	0.004
No	4/220(1.8%)	1		1	
Use of replants					
Yes	3/11(27.3%)	1		1	
No	14/342(4.1%)	0.114(0.27 – 0.476)	0.003*	0.153(0.023 – 1.025)	0.053
Utilization of ITN					
Yes	6/35(17.1%)	1		1	
No	11/318(3.5%)	5.77(1.99 – 16.752)	0.001*	3.51(0.973 – 12.681)	0.05

*** *P.value* <0.25, CI: Confidence interval, AOR: Adjusted odd ratio, COR: Crude odd ratio**

5.5. Prevalence of anemia and association with asymptomatic malaria

In a current study n=15 (4.2 %, 95 % CI [2.11, 6.29]) of participants were anemic, of this n=14 (93.3%) were mild anemic and n=1 (6.7%) were moderate anemic. There was no severe anemia were found. The minimum and maximum hemoglobin level was 7.5 and 24.6; while the mean hemoglobin level was 15.76. Among asymptomatic malaria positive participants n=3 (18 %) was had mild anemia, according to WHO standard. In this study a significant association was observed between asymptomatic malaria and anemia; the individuals that are positive for Plasmodium parasite have 5.8 times more likely to be anemic than those who are negative individual [OR=5.786 (1.465, 22.852), P=0.012] (Table 4).

Table 4. Univarat analysis of anemia Vs socio-demographic character and asymptomatic malaria infection in Mizan-Aman town; Ethiopia, February to April 2019

Variables		No anemic/ Total examined	COR(95% CI)	*P-value*
Gender	Male	7/164(4.3%)	1	
	Female	8/189(4.2%)	0.99(0.35,2.8)	0.99
Occupation	Government	3/40(7.5%)	1	
	Farmer	1/78(1.3%)	0.16(0.016,1.59)	0.118
	House wife	3/84(3.6%)	0.45(0.088,2.37)	0.351
	Merchant	3/23(13.0%)	1.85(0.34,10.02)	0.476
	Daily lobber	0/9(0.0%)	0	0.99
	House servant	1 /4(25%)	4.1(0.32,52.7)	0.277
	Student	4/115(3.5%)	0.44(0.095,2.078)	0.303
Monthly estimated income	< 650	4/114(3.5%)	0.412(0.088,1.93)	0.26
	650-1300	1/37(2.7%)	0.315(0.31,3.175)	0.327
	>1300	3/37(8.1%)	1	
Asymptomatic malaria	Negative	12/336(3.6%)	1	
	Positive	3/17(17.6%)	5.78(1.46,22.85)	0.012*

*** *P.value* < 0.05, CI: Confidence interval, COR: Crude odd ratio**

CHAPTER SIX

6. Discussion

Our study findings suggested the presence of asymptomatic malaria infection in the study settings as confirmed through either of one conventional malaria diagnosis techniques (microscopy and/or RDT). The overall prevalence of asymptomatic malaria was 4.8 % (17/353). None regularly used ITN, living in none IRS sprayed home and presences of mosquito breeding site were having a risk of asymptomatic malaria infection. On the contrary, the presence of asymptomatic malaria had a risk of anemia. The greatest proportion of asymptomatic malaria infection was detected in male sex groups 6.1% (10/164), age group ≥ 35 in 7.4% (5/68) and in shesheka kebele residence 7% (13/187).

Thus, our study finding was comparable with a study conducted in the Great Mekongi sub-region, Haite, and North Peruvian amazon that showed 5% (45), 3% (46), and 4.9% (47), respectively. Correspondingly, a study conducted in Ethiopia found comparable findings, in Dembia district which is 6.7 % (53), in Debre Elias East Gojam which is 4.8% (54) and in Benna Tsemay district which is 6.1 % (58). However, our study findings were relatively higher than a study conducted in eastern Myanmar, that showed 1.44% (48) of asymptomatic malaria infection; this difference might be due to the transmission intensity difference, their level of transmission was low; sampling technique difference, which was used convenient sampling technique**.**

Other studies conducted in Africa depicted low prevalence of asymptomatic malaria infection 1.0 % and 0.8%, in Zanzibar and Namibia, respectively (49,51); this discrepancy may be due to the difference of transmission intensity and study area altitude, our study area transmission intensity was moderate, whereas Zanzibar and Namibia where low transmission area, and altitude difference; also in Namibia vector controlling tools and treatment was improved and it became equivalent to pre-elimination phase. One survey conducted in Oromia regional state of Ethiopia showed a low prevalence of malaria 0.56 % (56). This difference might be due to study participants' difference; the majority of our study participants' age was greater than 15; whereas in Oromia regional state survey the participants were school children; this may be due to the fact that in an endemic area the children have ability of develop protective immunity to malaria

infection. Therefore, this may decrease the density of parasites and unable to be detected through conventional diagnostic techniques.

Whereas, some studies conducted across African countries showed a much higher prevalence of asymptomatic malaria as compared to this study's findings. In Gabon 18.8% (37) of asymptomatic malaria was revealed; this contrast might be due to the difference between the participants. Our study participants were recruited from the urban area and include all age groups, whereas in Gabon's study the participants were recruited from the different settlements, come from the malaria-endemic area and the transmission intensity of the area was perennial. Similarly, in Nigeria, two studies had shown a high prevalence of asymptomatic malaria 69.9% and 26 % (38,52), respectively. This may depict the participant and climate condition difference. In the study conducted at Abuja municipality, participants were from the malaria-endemic area, households were linked to malaria patients attending health facilities and the temperature was slightly high that may increase the sporogony cycle. In the other study conducted in Southwest Nigeria, participants were from the malaria-endemic area.

Likewise in Ethiopia, a much higher prevalence of asymptomatic malaria was found 18.4% in Armachio district and 29.8 % in Jimma town (55,57), respectively. This great difference may be due to the transmission intensity and the participant's differences. The Armachio district study area was hyperendemic malaria transmission and the participants were laborer migrants. Whereas, in Jimma town, enrolled participants were febrile and asymptomatic individuals so this may increase the chance of detecting more cases through conventional techniques. Similarly, a study conducted in West Arsi Zone showed 8.2 % (40), this contrast may be due to topography difference; the presence of manmade rainwater storage in other study areas may contribute to the continuous transmission of malaria through the breeding of mosquito vectors. Another study conducted in Arba-Minch town also found high prevalence which is 9.1% (59). This difference may be due to the study participants i.e. among pregnant women. This may be the fact that pregnant women had more susceptibility rates than other healthy individuals to malaria infection.

In the national malaria report, the dominant Plasmodium species was 60% due to *P. falciparum* and 40 % due to *P. vivax* (16). Likewise, other studies conducted in Ethiopia revealed similarly

much proportion of *P.falciparum* species than *P.vivax* (40,53,55,58). This might be due to *P.falciparum* species was the most widely distributed in Ethiopia. However, our finding revealed 70.5 % (12/17) due to *P.vivax*, and 29.5 % (5/17) due to *P.falciparum.* Other studies conducted in Ethiopia supports our finding by identifying a greater proportion of *Plasmodium vivax* species (57,59). This might be a result of the treatment i.e. the current national malaria treatment guideline is more focusing on the blood-stage of *P.falciparum* and besides that climatic variability in the study area has also effect in sporogony cycle of the parasite.

The correlation analysis of parasite density with the level of hemoglobin was not significantly correlated ($\alpha > 0.05$). The regression analysis of the current study showed that variables of sex, age in year, and locality or address were not significantly associated with asymptomatic malaria infection. However the greater proportion of malaria was detected in male sex 6.1% (10/164), ≥ 35 age group 7.4% (5/68), and in shesheka kebele residence 7% (13/187). This was similar to the study conducted in the North Gonder zone; males have a greater proportion than females and the age group was different i.e. adult ≥ 15 age; the greater proportion accounted in < 20 (53). The Debre Elias Gojam study similarly has shown a greater proportion of malaria in male sex and 25-34 age groups (54).

Similarly, in multivariate analysis previous history of malaria and self-medication have not significant association with asymptomatic malaria infection. Whereas the proportion of individuals with no history previous malaria infection was found to infected 7.1% than those who have the previous history 4.6%. This might be due to the fact that having the previous exposer to malaria infection help to develop acquired immunity i.e. anti-parasite, anti-immunity, and anti-disease due to this the parasite density and symptoms may decrease. Additionally, individuals who have not the habit of self-medication have more infection 6.3% than those who have habit 4.0 %, this might help to prevent the malaria parasite density a result of takings self-medication. However, in national malaria prevention and controlling strategy, an insecticide based vector control mechanism is a key strategy in the prevention and control of malaria transmission. Ensuring and maintain universal access of at-risk populations through the distribution of LLINs and IRS are the major vector controlling intervention (16). Our study confirmed that those who are regularly using ITN while the bedtime was 3.5 times protective for

malaria infection than those who didn't. Other studies conducted across in Ethiopia, also supported our results (53,54,56,59,66).

In a current study, the individuals those who living in none IRS sprayed home and present the proximity of stagnant water had more likelihood to be malaria positive than those who live in the sprayed house and absences of stagnant water around the home. Other studies conducted in Nigeria (38) and Gabon (37) also support this study results i.e. close proximity of stagnant water in residence are significant risk factors for asymptomatic malaria. Similarly, a study conducted in northwest Armachiho district (55) and North Gondar zone Dembia district, northwest Ethiopia (53), reveled both living in proximity of stagnant water and none IRS sprayed house was significantly associated with asymptomatic malaria; which was also similar in Oromia regional states study (56). This is because of the fact that environmental management is a key preventive tool for mosquito breeding and decrease transmmision.

The overall prevalence of anemia in a current study was 4.2 % (n= 15), among malaria positive individuals 18 % (n=3) had mild anemia. Another study conducted in Indonesia found a much greater prevalence as compared to this study; the prevalence was 32.8 % (62). This contrast may be due to the difference in parasite density and the numbers of asymptomatic malaria infected participants, in the current study infected individuals were low as compared to other studies. Also, a study conducted in Nigeria found a much greater prevalence of anemia, of those 28.5%, 45.5%, and 14.6% had mild, moderate, and severe anemia, respectively (63). This greater discrepancy might be due to the study population difference; our study was among the community whereas a study conducted in Nigeria was among health institutions those who come for other medical issues.

In Benishangul Gumuz Regional State, Ethiopia, the similarly much greater prevalence was found 73.76% (194/263) (64), this difference might be the fact that participant of the current study was among asymptomatic healthy participants, but another study was conducted among symptomatic individuals, <10 year and may be due to nutritional difference (particularly iron deficiency). A study conducted in Arba Minch Town, among pregnant women, has found the overall prevalence of anemia to be 34.6 % (118/341), among positive asymptomatic malaria 90 % (27.9/31) had anemia (59), this study highly different from our study these might be due to the

fact that physiological difference; the pregnant women require more nutrients especially irons, as a result of this individuals may become anemic.

The bivariate analysis has shown a significant association between asymptomatic malaria and anemia. This result agreed with other studies conducted elsewhere (59,62,63,67). This agreement may be the fact that the cause of anemia due to malaria is multifactorial; these may be due to the destruction of infected erythrocyte, impaired erythropoiesis, and the clearance of uninfected erythrocyte the result of parasite adhesive proteins through the lymphatic system.

Chapter seven

7. Conclusions and recommendations

7.1. Conclusion

The findings of this study showed asymptomatic malaria is a public health problem in a community of Mizan Aman town, it showed that (4.8 %, 95% CI= 2.6 - 6.99) of asymptomatic malaria cases. Low utilization of ITN, low coverage of IRS, and the presence of mosquito breeding sites in residence were having a significant association with asymptomatic malaria. Asymptomatic malaria infection was one of the risk factors for anemia in the current study, even though the cause of anemia is multifactorial, particularly nutritional deficiency.

7.2. Recommendations

In this study, conventional malaria diagnostic techniques were used, but it may not be truly determined thus asymptomatic low-density cases. Therefore, further studies including molecularly genotyping are required to determine the low density of sexual malaria parasite and immune system evaluation are required to a better understanding of the epidemiology of asymptomatic malaria infection. Regional health office and community health workers should scale up the coverage of ITN, IRS in study setting and communities should be engaged in environmental management to prevent the establishment of mosquito breeding sites.

7.3. Limitations of the study

The limitation of this study is that the prevalence of asymptomatic malaria parasites was determined by conventional diagnostic techniques; this may not truly detect a low density of asymptomatic malaria infection of sexual and asexual parasites in this study.

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Annex-I

JIMMA UNIVERSITY INSTITUTE OF HEALTH AND FACULTY OF HEALTH SCIENCES SCHOOL OF MEDICAL LABORATORY SCIENCES

Questionnaire

An interviewer guided questionnaire for assessment of socio-demographic data, socio-economic data and risk factor of malaria infection in selected site at Mizan-Aman, south-west Ethiopia.

Date of data collection_____ Kebele _____

PART ONE: **Socio-demographic and Socio-economics Data**

1. Age in year's _____
2. Sex □ Male □ Female
3. What is your educational status?
   - □ Illiterate
   - □ Only able to write and read
   - □ Primary school (1-8)
   - □ Secondary (9 - 12)
   - □ University/College
4. What is your occupation?
   - □ Gov.t /NGO employee
   - □ Farmer
   - □ House wife
   - □ Merchant
   - □ Daily laborer
   - □ House servant
   - □ Jobless
5. How much your monthly income (in ETB)? _____

PART TWO: **Risk Factors**

6. Do you use bed net (ITN)? □ yes □ No □ Sometimes
7. Do you use replant during bed time □ yes □ No
8. Have you ever been mosquito bite experiencing

   11.1. If the answer is yes for above question, where is your resting behavior?

   □ Indoor □ out door

9. Do you have history of fever? □ Yes □ No

12.1.If yes your answer for above question, what was the cause of fever ?

□ Malaria □ Other ----------

13. Have you ever been self-medication □ Yes □ No
14. Do you have previous malaria history? □ Yes □ No

PART THREE: Observation

15. Did sprayed antimalarial spray (IRS) □ Yes □ No
16. Is there stagnant water in your surrounding residence? □ Yes □ No
17. What type of House wall do you have □ Wood □ Brick □ Other _____
18. What type of House floor do you have □ Wood □ cemented □ muddy
19. What type of latrine do you have? □ Modern □ Open water carriage □ Other

PART Four: Laboratory Result

20. Status of plasmodium parasite with microscopy □ Positive □ Negative

20.1.If the answer is positive for the above question, which species are identified

□ *P.f* □ *P.v* □ Mixed

21. Hemoglobin level _____
22. RDT result □ *P.f* □ *P.v* □ Mixed

Date _____ sign _____

Annex-II

1. Venous blood collection procedure

2. Assemble the necessary materials and equipment.
3. Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol then apply the tourniquet on the 7.5 – 10 cm above the venipuncture site.
4. Grasp the back of the patient's arm at the elbow and anchor the selected vein by drawing the skin slightly taut over the vein insert the needle properly into the vein.
5. The tourniquet released the moment of blood starts drawing.
6. After drawing the required blood sample (3-4 ml), apply a ball of cotton to the puncture site and gently withdraw the needle and instruct the patient to press on the cotton.
7. The collected blood ejected in to heparinized vacationer tube and invert 8-10 the tube like figure 8.
8. Label the tubes with patient's name, hospital number and other information required by the hospital (before the patient leaves the collection area)
9. Re-inspect the venipuncture site to ascertain that the bleeding has stopped. Do not let the patient go until the bleeding stops.

2. Thick and thin blood smears procedure

1. Use sing slide for both thin and thick smears.
2. For thin film smear: one drop of whole blood on a clean slide, then clean slid spreader held at a 45° angle, toward the drop of blood on the specimen slide.
3. Wait until the blood spreads along the entire width of the spreader slide.
4. While holding the spreader slide at the same angle, push it forward rapidly and smoothly.
5. For thick film smear: Using the corner of a clean slide, spread the drop of blood in a circle in diameter of 1-2 cm.
6. Do not make the smear too thick or it will fall off the slide. (Able to read newsprint through it). Wait until the thin and thick films are completely dry before staining.
7. Fix the thin film only with methanol (100% or absolute) and let it dry completely before staining. The thick film should not be fixed.

3. Giemsa staning procedure

1. Using a Pasteur pipette, fix the thin film by carefully dropping methanol onto the thin film only.
2. Let the blood film dry in air on a drying rack or tray
3. Place slides for staining blood films face down on a curved staining tray or face up on a staining rack.
4. Pour stain slowly on or under the slide until the blood films are covered.
5. Set the timer to 10 minutes for the staining.
6. Gently flush all the stain from the slides by dropping clean water over it.
7. Allow the slides to air-dry.
8. Wipe the back of each slide clean and place it in a draining rack for the preparation to air-dry
9. Examine with oil immersion.

4. RDT procedure

1. Dispatch the test kit and labile the patient identification number on the caste.
2. Using provided pipette or micropipette, 5 $\mu\ell$ of whole blood drop into the 'S' well.
3. Add 60 $\mu\ell$ assay buffer solution (3 drops for vial type or 2 drops for bottle type) into the "A" well. Start a timer.
4. Read result in 20 minutes.
5. Interpretation of the test result as following,
   - ❖ Invalid, when a line does not appear next to “CON”, should be repeated using a new cassette.
   - ❖ Negative, the presence of a line next to “CON” with no other line indicates a negative result
   - ❖ *P.falciparum* positive, the presence of two line (one line in the result window next to “CON” and another line in the result window next to “*Pf*”.

- *P.vivax* positive, the presence of two line (one line in the result window next to "CON" and another line in the result window next to "*P.v*" indicates a positive result for *P.vivax.*
- *P.falciparum and P.vivax* positive, the presence of three line (3 line in the result windo next to "CON", "*Pf"and* "*P.v*") indicates a positive result for *P.falciparum* and *P.vivax.*

5. HemoCue® Hb 301 System procedure

1. EDTA blood sample fill the microcuvette in one continuous process. Do NOT refill! If a second sample is to be taken, fill a new microcuvette from a new drop of blood. This should not be done until the measurement of the first sample is completed.
2. Wipe off excess blood from the outside of the microcuvette with a clean, lint-free wipe. Do not touch the open end of the microcuvette.
3. Look for air bubbles in the filled microcuvette. If present, discard the microcuvette and fill a new microcuvette from a new drop of blood. Small bubbles around the edge can be ignored.
4. Place the microcuvette into the cuvette holder and start measurement as soon as possible but no later than 40 seconds after filling the microcuvette by gently pushing the cuvette holder to its measuring position.
5. After ≤3 seconds, the hemoglobin value is displayed. The result will remain on the display as long as the cuvette holder is in the measuring position. Do not remeasure the microcuvette. When operating on battery power, the analyzer will automatically turn off after approximately 5 minutes.

Annex –III

Information sheet and consent form

JIMMA UNIVERSITY INSTITUTE OF HEALTH AND FACULTY OF HEALTH SCIENCES SCHOOL OF MEDICAL LABORATORY SCIENCES

1. English version

Name of organization

Jimma University, Institute of health, School of Medical laboratory sciences

Topic

Prevalence of asymptomatic malaria and associated risk factors in mizan-aman town, Ethiopia.

Investigator

Mr. Kassahu Demelash (BSc)

Advisors

Dr. Ahmed Zeynudin (PhD)

Mr. Abdissa Biruksew (PhD fellow)

Prof. Delenasaw Yewhalaw (PhD)

Purpose of the research

The purpose of this research is to determine the magnitude of asymptomatic malaria and risk factors at mizan-aman town, south west Ethiopia.

Voluntary of the study participants

The right of study participants to be enrolled or to refuse respected. All study subjects were participating freely after being informed about the study purpose. They will have the right to refuse answering questions, giving blood and withdraw from the study at any time. We will not expect from you to give a reason to stop the study.

The risks and benefits

The research will be carried out with minimum or no risk for the subjects. There will be no discomfort during sample collection. We would fully respect the individual, the culture and the society. Moreover, all who had the malaria parasite will be treated by linking to health extension worker according to national guide line treatment with considering the result.

Confidentiality

Ensured by making laboratory results record format anonymous and by publishing research findings in a way that were not relate to the study subject.

Contacts

If you have any question, suggestion comments or anything that is not clear; please contact us;

1. Mr. Kassahun Demelash (BSc)
2. Dr. Ahmed Zeynudin (PhD)
3. Prof. Delnaesaw Yewhalaw (PhD)
4. Mr. Abdissa Bierukisew (PhD fellow)

Finally, we would like to say thank you for taking time to hear the information given and willing to participate the study. If you are clear with the information provided and agree to participate, please sign the next page on the consent form.

Consent form

Prevalence of asymptomatic malaria and associated risk factors in mizan-aman town, Ethiopia.

1. English version

I, the undersigned individual, am oriented about the objectives of the study. I have informed that all of my information will be kept confidential and used solely for this study. In addition, I have been well informed that my name will not be asked and unique identification is not required. If I want to withdraw from the study anytime along the process, I will not be obliged to continue or give reasons for doing so. However, my agreement to participate in this study is with the assumption that, the information and the specimen that I provide will help greatly to the determined asymptomatic malaria cases and hemoglobin levels.

Signature;_____ Date; _____

ጅማ ዩኒቨርስቲ ጤና ሳይንስ ኢንስቲቲዩት ፣ላቦራቶሪ ሳይንስ ት/ት ክፍል

2. ስለጥናቱ ኢንፎርሜሽን በአማርኛ ግልባጭ (Amharic version)

ድርጅት

ጅማ ዩኒቨርስቲ ፤ ጤና ሳይንስ ኢንስቲቲዩት፤ ላቦራቶሪ ሳይንስ ት/ት ክፍል

የጥ ናቱ ርዕስ

በወባ ትንኝ አማካኝነት የሚተላለፈውን የወባ በሽታ ስርጭት መመርመር ፣ እና ስርጭቱን የሚያባብሱ ነገሮችን ማእሰስ በሚዛን አማን ከተማ በተመረጡ ቀበሌዎች ላይ ማጥና ።

ተመራማሪዎች

1. አቶ ካሳሁን ደመላሽ (ተመራማሪ)
2. ዶ/ር አህመድ ዘይኑዲን (አማካሪ)
3. ፕሮፌሰር ድልነሳው የኃላው (አማካሪ)
4. አቶ አብዲሳ ብሩክ (አማካሪ)

መብት

አንተ/ቺ በዚህ ጥናት በመሳተፍህ/ሽን ለየት ያለ የምታገኝዉ/ኚዉ መብት የለም ። ነገር ግን የምታገኘው/ የምታገኚዉ ጥቅማ ጥቅም ካለ በማንኛውም መንገድ የማይገድብብህ/ሽ ነው።

ጥቅም

በጥናቱ ውስጥ በመሳተፍህ/በመሳተፍቸሽ የምታገኘው/ የምታገኚዉ መብት የለም ነገር ግን ጥቅማጥቅሞች ባይኖሩም በምርመራ ወቅት በምትሰጠው ናሙና ውስጥ የወባ ፓራሳይት ከተገኘበት በነፃ መድሀኒት እንድታገኝ/ኚ ከጤና ኤክቴንሽን ባለሞያዎች ጋር በማገናኘት ትክክለኛውን መድሀኒት እንድታገኝ/ኚ አደርጋለሁ ።

ሚስጢራዊት

ሁሉም ስለጤንነትህ/ሽ የሚጠቅሱ የምርመራ ውጤቶች ሚስጢራዊነታቸው በሚገባ የሚጠበቅነው ። በማንኛውም ሪፖርት ወይም ህትመት አትታወቅም/ቂም።

የሚያደርሰዉ ጉዳት- ይህን በማድረግህ/ሽ ምንም አይነት ጉዳት የሚደርስብህ /ሽ የለም።

የስምምነት ማረጋገጫ (Amharic version)

ይህ ስምምነት የተዘጋጀው በሚዛን አማን ከተማ በተመረጡ ቀበሌዎች ላይ ምልክት አልባ የወባ በሽታ ስርጭት መመርመር ፣ እና ስርጭቱን የሚያባብሱ ነገሮችን ማሰስ በተጨማሪም ሂሞግሎቢን መጠንን ለማወቅ ለሚደረግ ጥናት የተዘጋጀ የስምምነት መግለጫ ቅፅ ነው።

ማብራሪያ

እኔ ስሜ ሳይጠቀስ በመለያ ኮድ ብቻ የምለየዉ የምርምሩ ተሳታፊ ስለምርምሩ በቂ ገለጻ ክተደረገልኝ በኃላ በደሜ ውስጥ ለሚገኝው የውባ ፓራሳይት እና ሂሞግሎቢን መጠንን ለማወቅ ምርመራ እንዳደርግ በተጠየኩት መስረት ደም እንድሰጥ ተጠይቂያለሁ። እነዚህ ፓራሳይቶች እኔ በምሰጠው ናሙና ውስጥ የሚገኙ ከሆነ አስፈላጊው ህክምና የሚሰጠኝ እንደሆነ፣ ናሙናውን በመስጠት ምንም አይነት ጉዳት የማይመጣብኝ መሆኑን፤ የምርመራው ውጤት በሚስጥር እንደሚጠበቅ ተገልፆልኛል። ከሁሉም በላይ በጥናቱ ላይ መሳተፍ በፍቃደኝነት ላይ የተመሠረተ መሆኑና ለመሳተፍ ፍቃደኛ ከመሆኔ በፊት እንዳስብበት በቂ ጊዜ ተሰጥቶኛል ።

ስለዚህ በጥናቱ ለመሳተፍ የወሰንኩት ስለሁኔታው በሚገባ ከተረዳሁ በኋላ በጥናቱ ሂደት ውስጥ በፍቃደኝነት ለመሳተፍ ተስማምቻለሁ ።

ፊርማ _____ ቀን _____

Declaration Sheet

I, the undersigned, MSc Medical parasitology student declares that this thesis is my original work in partial fulfillment of the requirement for the degree of master science in Medical parasitology. Where others work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Name of principal investigator

Mr. Kassahun Demelash (MSC Candidate) Signature _____ Date_____

Approved by my advisors;

Dr. Ahmed Zeyunidn (Associate professor, MSc, PhD) Signature _____, Date_____

Prof. Delnesaw Yewhalaw (PhD, Professor) Signature _____ Date_____

Mr. Abdissa Berukisew (Assistant professor, MSc, PhD fellow)

Signature _____Date_____