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Studies on Biochemical and Hematological Parameters among Male Population in a Severe Malaria (*Plasmodium falciparum*) Infested Area of Gambella Region, in South Western Ethiopia.

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This is to certify that the dissertation prepared by Hadera G/krstos entitled: **Studies on Biochemical and Hematological Parameters among Male Population in a Severe Malaria (*Plasmodium falciparum*) Infested Area of Gambella Region, in South Western Ethiopia**, 2016/17 is Submitted in partial fulfillment of the requirements for the degree of "Master of Science in Medical Biochemistry" in the department of Medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper entitled **Studies on Biochemical and Hematological Parameters among Male Population in a Severe Malaria (*Plasmodium falciparum*) Infested Area of Gambella Region, in South Western Ethiopia**, is my original work and has not been presented for a degree in any other university and that all sources of materials used for the research have been properly and suitable acknowledged.

Hadera G/krstos Kidu

Signature_____________________

Date________________________

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LIST OF ABBREVIATIONS AND ACRONYMS

BMI	Body Mass Index
CBC	Complete Blood Count
EDTA	Ethylene Diamine Tetra Acetic acid
FBC	Full Blood Count
Fl	Femto litere
FMOH	Federal Ministry of Health
Hb	Hemoglobin
Hct	Hematocrit (Relative Volume of Erythrocytes)
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MV	Mechanical Ventilation
OPD	Outpatient Department
PCV	Packed Cell Volume
PCR	Polymerase chain reaction
PF^{+4}	>10 parasites per high power field (++++)
Pg	Pico gram
PLT	Platelet
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
WHO	World Health Organization

OPERATIONAL DEFINITIONS

- **Anemia:** - A reduction in the number of circulating red blood cells or in the quantity of hemoglobin.

- **Fasting blood glucose**:- measures blood glucose after an individual have not eaten for at least eight hours.

- **Hematological parameters**:- Hematological parameters are measurable indices of the blood that serve as a marker for disease diagnosis.

- **Hemoglobin:**- this is a conjugated protein with high affinity for oxygen and low affinity for carbon dioxide within red blood cells.

- **Parasitemia**:**-** The presence of parasites in the blood. In malaria, a measure called the parasitemia index reflects the severity of the disease

- ***Plasmodium falciparum****:* - is an intracellular protozan that may cause severe forms malaria and the most pathogenic and the cause of most complications of malaria.
- **Protozoa:**- Are unicellular, eukaryotic organisms**.**

- **Severe malaria**: - is acute malaria with signs of organ dysfunction and/or high level of parasitemia and demonstrable malaria parasitemia in a peripheral blood sample or a medical emergency requiring immediate hospitalization with prompt initiation of appropriate parenteral therapy to rapidly reduce and eliminate parasitaemia.

- **Thrombocytopenia:-** A condition in which there is an abnormally low number of platelet in the circulating blood.

ABSTRACT

Malaria is a blood disease caused by the bite of *Anopheles* mosquito. It is also a major health problem in developing countries accounting for 2-3 million deaths per year. The burden of malaria is increasing especially in sub-Saharan Africa because of drug and insecticide resistance, social and environmental changes, creating a major challenge in providing patients with this infection with effective malaria chemotherapy. Severe malaria affects almost all blood components and is a true hematological infectious disease. Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications and they play a major role in malaria pathogenesis. Hypoglycemia is a frequently encountered complication in severe *Plasmodium falciparum* malaria, which is usually ascribed to decreased glucose use and impaired glucose production caused by the inhibition of gluconeogenesis. The Gambella People's Regional State is located at South West of Ethiopia and malaria is the leading cause of morbidity and mortality in this region. The objective of this study was to investigate the effect of severe *Plasmodium falciparum* malaria on some biochemical and hematological parameters in Gambella region at Gambella General Hospital on adult males aged from 18-55. Hyperparasitemia in the adult males was considered as an important criterion for selection of individuals with severe malaria. Fasting blood glucose and hematological parameter (RBC, Hb, Hct, MCV, MCH, MCHC and PLT) were measured in patients ($n = 60$) and control subjects ($n = 40$). The results were analyzed using student t-test and indicated significant difference in the hematological parameters between the malaria infected and non-infected subjects ($p < 0.01$). Adult males with severe malaria had significantly decreased levels of RBC, Hb, Hct and PLT also a significantly decreased level of fasting blood glucose ($p < 0.01$) but the level of MCV, MCH and MCHC were not significantly decreased when compared with the control group ($p > 0.05$). Hb and fasting blood glucose are positively correlated with demographic indicators and PLT negatively correlated with the demographic indicators in the study groups and control groups. The study concluded that investigation of biochemical parameters and hematological parameters showed certain changes of a sub-clinical pattern and severe *Plasmodium falciparum c*an cause significant hematological changes with high incidence of thrombocytopenia and anemia. Consideration of these changes in adult males with severe *Plasmodium falciparum* malaria might effectively reduce morbidity and mortality.

Key words: - Fasting blood glucose, severe malaria, *Plasmodium falciparum* parasites, Hematological parameters, Gambella, Ethiopia.

1. INTRODUCTION

1.1. Back ground information

The term malaria originated from Italian word “mal” and “aria”: meaning bad air and the disease was called ague or mesh fever due to its association with swamps in humid regions of the world. It is an ancient disease that has plagued humans throughout history (Ani, 2015). It is a term used for acute or chronic infection caused in man or other vertebrates such as mice and donkeys. It is also a major public health problem in the world whose transmission is influenced by several inter-linking factors like climate, environment and demography. According to World Health Organization 2012 estimation, there were about 209 million cases of malaria in the world and Sub-Saharan Africa was the most affected region with estimated deaths of 627,000. Malaria remains one of the most pressing health problems in the world with an estimated 300-500 million cases annually of which 90% occurs in Sub Saharan Africa. The majority of malaria cases in Africa are due to *Plasmodium falciparum*, which associated with mortality and morbidity (Yaya, 2011, Sissay and Gebreegziabhier, 2015, Sirak *et al*., 2016).

People living outside areas of endemic malaria are more susceptible to severe complicated forms of the disease. This can be due to lack of specific immune response against *Plasmodium*, which depends on prolonged and repeated antigenic exposure. Another factor to be considered is the risk of delay in diagnosis, as outside endemic areas, physicians are less familiar with the management of malaria (Dotrário *et al. 2016)*.

Malaria can affect single or many organs with different levels of severity, which can be determined as neurologic and renal dysfunction, hematologic, cardiovascular and respiratory dysfunction as well as hepatic and metabolic dysfunction (Godse, 2013). Generally most severe malaria complications occur in non immune subjects with *Plasmodium falciparum* malaria and involve central nervous system (cerebral malaria), pulmonary system (respiratory failure), renal system (acute renal failure) and/or hematopoietic system (severe anemia). As the progression to these complications can be rapid and severe malaria is a potentially a fatal disease. Any malaria patient must be assessed and treated rapidly and frequent observations are needed to look for early signs of systemic complications (Bartoloni and Zammarchi, 2012).

Malaria is an infectious disease/infection caused by a protozoan called *Plasmodium* species and caused by five human *Plasmodium* species (i.e. *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi* (Geleta and Ketema, 2016). It also a major health problem in developing countries accounting for 2-3 million deaths per year like Ethiopia with wide-ranging hematological and biochemical alterations (Kosiyo, 2016). Therefore, the aim of this study was to assess malaria parasitemia and its association with biochemical and hematological parameters in severe malaria caused by the *Plasmodium falciparum*.

Malaria is a disease that can be transmitted to people of all ages. The malaria parasite has a complex life cycle and transmitted to human by the bite of an infected female *Anopheles* mosquito which harbours the parasite. It is mainly caused by various species of *plasmodium* parasite (Al-Salahy *et al*., 2016). The major complications are caused by *Plasmodium falciparum* and *Plasmodium vivax* with *Plasmodium falciparum* being the more virulent. They are prevalent in all malaria areas in the country and their relative composition generally is 60% and 40% of the malarial cases, respectively. *Plasmodium falciparum* is the most causative agent of severe malaria in humans resulting in high mortality and morbidity. Based on Ethiopia"s Federal Ministry of Health report in 2009/2010, severe malaria infection was the leading cause of outpatient visit admissions, accounting for 14% and nearly 9% of admissions and it was among the ten leading causes of inpatient deaths in children and pregnant mothers in Ethiopia (Yaya, 2011, Sirak *et al*., 2016).

In Ethiopia, malaria transmission is largely determined by altitude and climate as affected by Indian Ocean conditions and global weather patterns. Most of the malaria transmission occurs between September and December, after the main rainy season from June to August. Certain areas largely in the western and eastern parts of the country experience a second less extent malaria transmission period from April to May, following a short rainy season from February to March (Yaya, 2011, Alemu *et al*., 2011). This corresponds with major harvesting season with serious consequences for the subsistence Economy of Ethiopia‟s country side and for the nation in general. Malaria also poses no risk to in habitants at altitudes above 2000m, mostly in the Northern and Eastern Highland. Regionally, all of Gambella, most parts of Benishangul-Gumuz, the lower elevations of the Northern and Eastern Highlands are also known areas of malaria endemicity. The western, central and eastern highlands, as well as the highland-fringe areas along the Rift Valley are especially vulnerable to epidemics. In all, more than half of the population lives in epidemic environmental exposure areas (Temesgen, 2015).

Malaria is also major obstacle to socioeconomic development in Ethiopia. Around 75% of the geographic area of the country has malaria transmission (defined as areas < 2,000m) with 68% (i.e.54.2 million) of the country total population living in these areas (Yaya, 2011, Jima et al., 2010). According to FMOH reports approximately 70,000 people die of malaria each year in Ethiopia (Worku *et al*., 2014, Watiro and Awoke, 2016). Due to the unstable and seasonal pattern of malaria transmission the protective immunity of the population is generally low and all age groups are at risk of infection and disease. Some small-scale studies have documented on malaria parasite prevalence in Gambella is between 10.4–13.5%. In addition to seasonal pattern of malaria transmission in Ethiopia especially in Gambella region anthropogenic factors are also contributing to the spread of malaria. These include population growth and movements, urbanization, water development schemes, agricultural development, conflicts and improper use of drugs and the attendant consequences of the emerging drug-resistant malarial parasites, deforestation poor housing, lack of proper sanitation, poor drainage of surface water, weak health services and wide-spread economic unequal, which independently or together facilitate malaria transmission and establishment of new settlements in previously unsettled areas. These allow making comfortable for the proliferation of mosquitoes that prefer human habitation to natural settings (Bayissa, 2007, Yaya, 2011, Alemu *et al*., 2011).

Studies have revealed that hematologic and biochemical changes occur in malaria infected blood and there are common complications associated with this disease. Malaria is known to cause hemolytic anemia as major hematological disorder. Hematological alterations are some of the most common complications that cause changes in major cell lines such as red blood cells and platelets (Imoru *et al*., 2013). With regard to the association between blood cells and malaria infection, analysis of hematological parameters indicates increased predictive value (Kimbi *et al*., 2013). Malaria can affect any organ or system and the hematological system being the most affected one. The majority of described alterations occur on the red cell line and platelets, causing anemia and thrombocytopenia (Martinez-Salazar, 2014). In spite of causing complication and malaria pathology, hematological changes such as anemia and thrombocytopenia have been reported but the degree of alterations varies according to the level of malaria endemicity, nutritional status, background hemoglobinopathy, density of parasite, socio-demographic factors and malaria immunity (Al-Salahy *et al*., 2016).

The extent of biochemical and hematological parameters changes during severe malarial infection and their underlying biological mechanisms in level of parasitemia remain unclear. Because of this knowing about malaria and its association with biochemical and hematological parameters could be important for management of malaria-infected patients and better understanding in this relationship is crucial in the development of a potential drug. Therefore, the present study aimed to examine the association of biochemical and hematological changes, assess malaria parasitemia and their diagnostic values in people infected with severe malaria among adult males in Gambella General Hospital, South West of Ethiopia. Hematological parameters including RBCs, Hb, PLT, Hcr, MCV, MCH and MCHC level of people infected with severe malaria were compared with uninfected people and to demonstrate the impact of severe *Plasmodium falciparum* malaria as well as different parasite densities on blood cell parameters in severe malaria patients.

2. LITERATURE REVIEW

Malaria is a tropical protozoan disease transmitted through female *Anopheles* mosquitoes. It is mainly caused by various species of *plasmodium* parasite (Kamble, 2011). It also one of the most widespread human parasitic diseases ranking first in terms of its socioeconomic and public health importance in tropical and subtropical region of the world, especially in sub-Saharan African and South East Asian countries (Worku *et al*., 2014). The *Plasmodium falciparum* causes the vast majority of the burden of malaria mortality is in sub-Saharan African countries because of drug and insecticide resistance and social and environmental changes. The high mortality rate from *Plasmodium falciparum* is due to its ability to induce severe malaria and in some cases, multiple organ dysfunction. The clinical presenting symptoms and mortality patterns of severe malaria of *plasmodium faliciparum* vary widely according to the geographical setting and transmission intensity (Abdallah *et al*., 2013).

Malaria is one of the most serious vector borne diseases transmitted by the bite of infected *Anopheles* mosquitoes which carry the *Plasmodium* parasites. It also occasionally transmitted through blood transfusion. If the person has malaria some of the parasites in the blood will be sucked into the mosquito. The malaria parasites multiply and develop in the mosquito. After 10-14 days they are mature and ready to be passed on to someone else. If the mosquito now bites a healthy person the malaria parasites enter the body of the healthy person. The parasites are transported in the blood stream to the victim's liver where they multiply and then re-enter the blood stream. The malaria parasites can multiply 10 times every 2 days destroying red blood cells and infecting new cells throughout the body. The person infected by the mosquito bite will become ill with malaria symptoms appearing anywhere from about a week to several months after infection but usually in 7-21 days average (Okwa, 2012, Temesgen, 2015, Kosiyo, 2016).

Altitude and climate (mainly rainfall and temperature) are the most important determinants of malaria transmission in the country although change in settlement patterns, drought and migrations could contribute a lot for the spread. Rainfall also influences malaria transmission by providing mosquito breeding places and increasing humidity, which improves mosquito survival rates. Transmission usually occurs at altitudes below 2000m above sea level. Likewise, it has been reported that although elevation is important factors such as rainfall, temperature and humidity levels play an important role in determining its intensity. Even, the relationship between malaria transmission and the rainfall is further complicated by temperature, humidity and the movement of people between lowland and highland areas (Bayissa, 2007, Yaya, 2011).

Changes in temperature, rainfall and relative humidity due to climate change are expected to influence malaria directly by modifying the behavior and geographical distribution of malaria vectors and by changing the length of the life cycle of the parasite. Climate change is also expected to affect malaria indirectly by changing ecological relationships that are important to the organisms involved in malaria transmission (the vector, parasite and host). Recent evidence shows that changes in temperature and precipitation have already changed the distribution and behavior of the vector (Alemu *et al*., 2011).

2.1. Hypoglycemia in adults of severe malaria

Hypoglycemia in severe *Plasmodium falciparum* the occurrence of malaria in adults is due to mal-absorption of glucose from intestine. Adults with severe malaria had greatly reduced absorption capacity for sugar transport both actively and passively. Most of the patients have hypoglycemia before quinine administration. This suggests that other causes may also be responsible for hypoglycemia (Chimalizeni *et al*., 2010). It contributes to nervous system dysfunction. Malaria induced hypoglycemia is often present at admission and response to glucose is unimpressive while quinine induced hypoglycemia usually develops 24 hours after treatment and response to glucose administration is dramatic. Blood glucose should be monitored as the signs may be very subtle. Hypoglycemia must be excluded in all patients with an altered mental state and in those who present with convulsions (Gomes *et al*., 2011).

Hypoglycemia is one of the tricky complications of *Plasmodium falciparum* malaria and may often go unnoticed, adding to the morbidity and mortality. It also one of the defining features of severe malaria according to WHO guidelines and indicates a poor prognosis. Hypoglycemia occur both on presentations to hospital and during the course of admission in an individual with severe malaria. The etiology is incompletely understood and is likely to be multi-factorial.

Depletion of glucose stores due to starvation, parasite utilization of glucose, cytokine-induced impairment of gluconeogenesis have been implicated (Ogetii *et al*., 2010).

In clinical practice different blood glucose thresholds are used to define hypoglycemia. Studies have shown that hypoglycemia < 2.2 mmol/l is associated with poor outcome) and this complication is incorporated within the case definition of severe malaria in adults. In conscious patients hypoglycemia may present with classical symptoms of anxiety, breathlessness, a feeling of coldness, diaphoresis, tachycardia and lightheadedness and signs of autonomic over activity (sweating or „gooseflesh‟). More severe signs include coma, deteriorating consciousness, abnormal posturing (decelerate or decorticate rigidity, muscle spasms, pouting, stertorous breathing and opisthotonos and generalized convulsions (Medana *et al*., 2002).

Plasmodium falciparum induced reduction in the blood glucose could be due to the invasion of the liver cells by the malaria parasite which can cause organ congestion, sinusoidal blockage and inflammation of pancreatic cells. It is common in patients with severe malaria and may lead to seizures and coma. The pathophysiology of adult hypoglycemia is usually associated with hyper-insulinemia which may result from pancreatic cell stimulation and *Plasmodium* species derived factors. Generally hypoglycemia is common in patients with severe malaria occurs due to the following factors like; increased consumption of glucose by the host and the growing parasites, stimulation of pancreatic insulin secretion by drugs like quinine, increased peripheral requirement of glucose consequent upon anaerobic glycolsis, increased metabolic demands of febrile illness, obligatory demand of parasites and failure of hepatic gluconeogenesis and glycogenolysis (parasites consume up to 70 times as much glucose as uninfected cells) (Patel *et al*., 2003, Gomes *et al*., 2011).

2.2. The life cycle of malaria parasites

When a female *Anopheles* mosquito ingests the blood of a person or animal with malaria parasite in the circulation, the asexual forms of the parasite are digested together with the red blood cells while the mature sexual cells (gametocytes) undergo further development. In the stomach of the mosquito the gametocytes develop into macrogamete and microgamete which are female and male gametes respectively after ex-flagellation. The macrogamete is fertilized by one microgamete to form a zygote (ookinate). This penetrates the cell wall of the mosquito and lies between the epithelium and the basement membrane (Okwa, 2012, Ani, 2015).

The parasite has a complex life cycle in which it undergoes several transformations (Fig 1). Following sexual reproduction in the mid gut of the female *Anopheles* mosquito, haploid sporozoites accumulate in the salivary glands of the vector and are transmitted to the human host during a blood meal. Sporozoites migrate to the liver and replicate within hepatocytes for approximately one week. Following this clinically silent liver stage, merozoite progeny exit into the bloodstream and initiate the erythrocytic replication cycle, during which all clinical manifestations of malaria infection occur. Merozoite invasion of red blood cells yields early ring forms, followed by growth and replication to produce mature schizont forms. Schizont rupture releases daughter merozoites that reinitiate the cycle. Some parasites develop into sex cells termed gametocytes, which can be acquired by the vector during feeding (White *et al*., 2014).

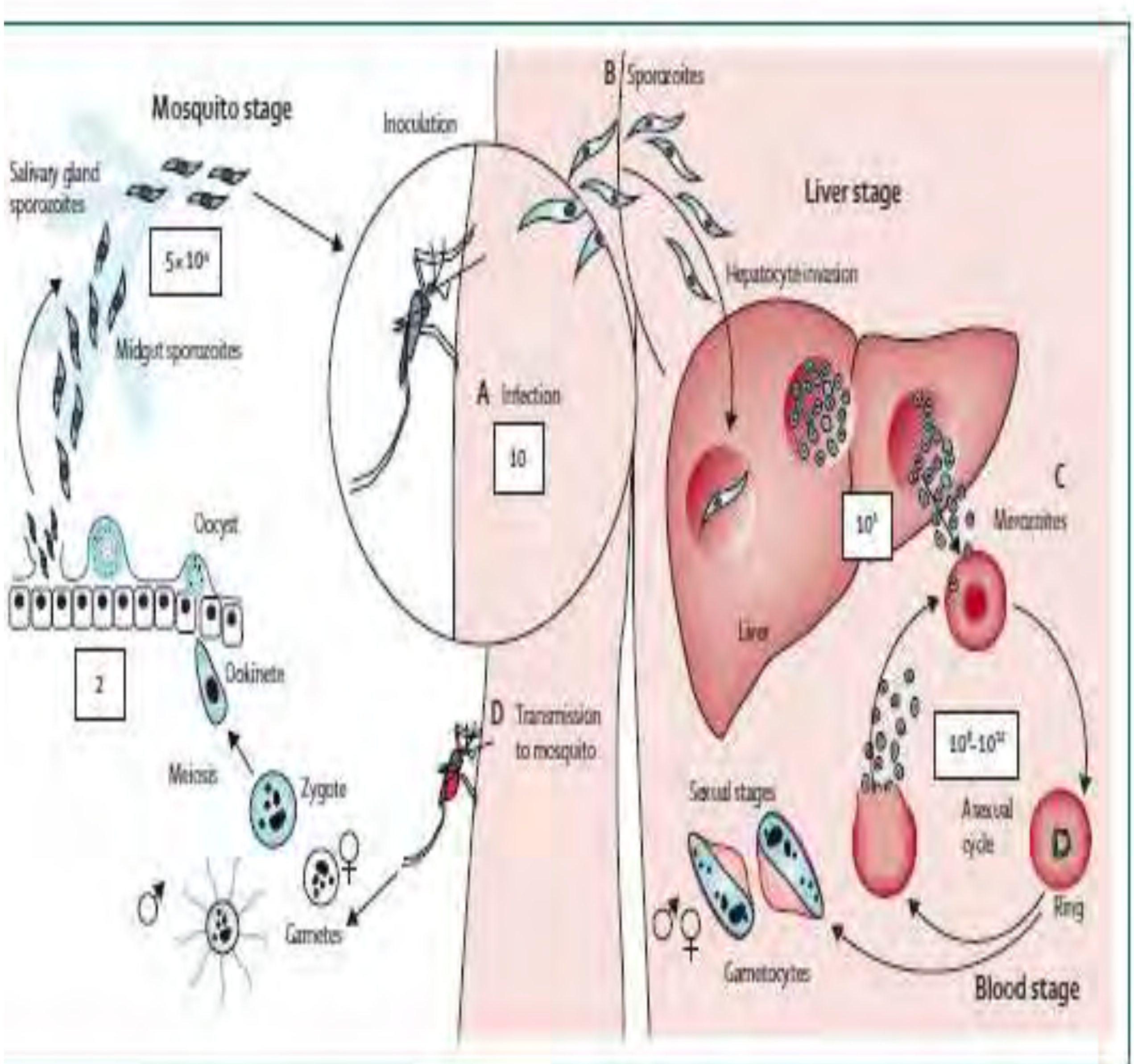

Figure *1:* Lifecycle of *Plasmodium falciparum* in the human body and in the females *Anopheline* mosquito (White *et al*., 2014).

The cycle begins with inoculation of motile sporozoites into the dermis **A**; magnified, which then travel to the liver **B**; each sporozoite invades a hepatocyte and then multiplies. After about a week, the liver schizonts burst, releasing into the bloodstream thousands of merozoites that invade red blood cells and begin the asexual cycle **C**. Illness starts when total asexual parasite numbers in the circulation reach roughly 100 million. Some parasites develop into sexual forms (gametocytes). Gametocytes are taken up by a feeding anopheles mosquito **D**, and reproduce sexually, forming an ookinete and then an oocyst in the mosquito gut. The oocyst bursts and liberates sporozoites, which migrate to the salivary glands to await inoculation at the next blood feed. The entire cycle can take roughly one month. Estimated numbers of parasites are shown in boxes a total body parasite burden of 10^{12} corresponds to roughly 2% parasitaemia in an adult.

Plasmodium species, the causative agents of malaria that can infect a wide range of hosts, including birds, reptiles and mammals. While malaria transmission was once widespread globally, it is now mainly limited to tropical and sub-tropical regions. Malaria transmission from an infected human host to a female *Anopheles* mosquito is mediated through highly specialized sexual-stage parasites, i.e., gametocytes. The gametocytes of *Plasmodium falciparum* hold a prominent place in the history of malaria. Transmission of malaria is affected by climate and geography and often coincides with the rainy season. Generally it can be transmitted to man through three known ways which are vector transmission, blood transmission and congenital transmission (Sumbele *et al.,* 2016, Geleta and Ketema, 2016).

Mostly, malaria is transmitted by female *Anopheles* mosquitoes a number and type of which determine the extent of transmission of infection in a given area. The transmission of malaria requires high environmental temperature and collected water body both, which are ideal conditions for breeding of mosquitoes. Therefore, transmission is common in lowlands during rainy season especially with migration of non immune individuals to these areas. The three most efficient species are *Anopheles gambiae, Anopheles arabienses* and *Anopheles funestus.* The transmission of malaria involves the transfer of the parasite from mosquitoes to man as much as from man to mosquitoes. The bite of an *Anopheles* mosquito carrying sporozoites within its salivary glands involves three distinct but linked processes. These are the penetration and probing by the mouth parts into the skin, the injection of saliva containing sporozoites and blood feeding. Sporozoites injected into the skin during probing seem to be the only ones capable of reaching the liver of the host. Sporozoites injected into venous blood during feeding have no effect on the parasitic cycle as they are immediately re-ingested by the mosquito in its blood feed (Temesgen, 2015, Ani, 2015).

Malaria risk map from annual parasite incidence analysis is shown in Figure 2, showing areas with malaria transmission risk by annual parasite incidence classified as high (>100), medium (5-99.9), low (0.1-4.9) and malaria free areas with the highest malaria transmission risk as stratified by district annual parasite incidence appear to be largely in the lowlands and midlands of the western border with South Sudan and Sudan, with some other high transmission areas in or near the Rift Valley (that extends from the southwest of the country to the northeast). Many densely populated highland areas were newly classified as malaria-free according to annual parasite incidence including the city of Addis Abeba (President‟s Malaria Initiative Ethiopia Malaria Operational Plan FY, 2015)

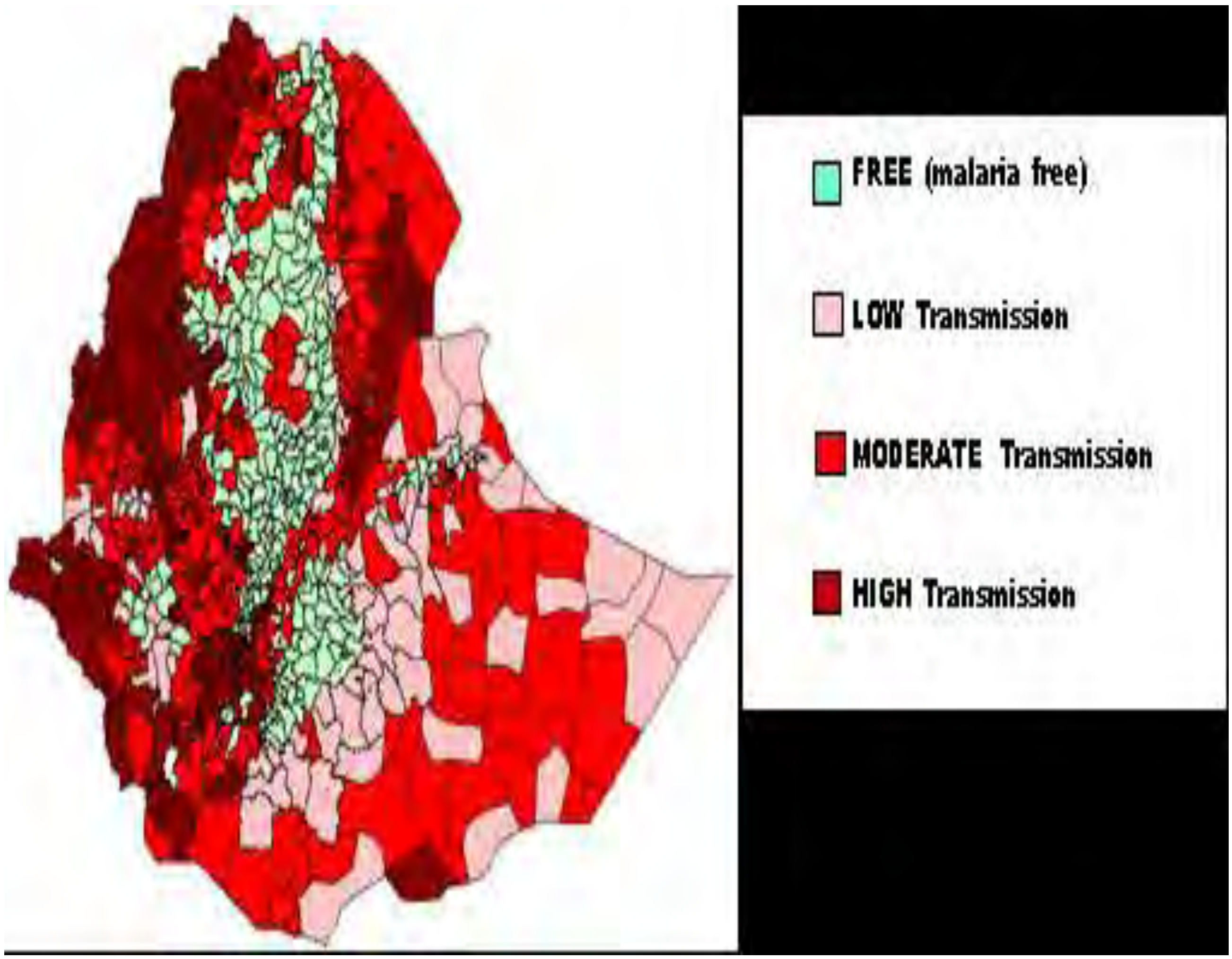

Figure 2: Malaria risk map of districts by annual parasite incidence, Ethiopia, 2014 (President‟s Malaria Initiative Ethiopia Malaria Operational Plan FY, 2015).

2.3. Vector Transmission

There are about 400 different species of *Anopheles* mosquitoes transmitting malaria of which only about 70 species have been definitely incriminated as vectors of human malaria (Bayissa,, 2007). Of these, the *Anopheles gambiae* complex and *Anopheles funestus* are the most efficient vectors for *Plasmodium falciparum* transmission; the highest rates of sporozoite development are in *Anopheles gambiae,* the species that is widespread throughout tropical Africa. The major malaria vector known in Ethiopia is *Anopheles arabiensis*. In some areas *Anopheles pharoensis*, *Anopheles funestus* and *Anopheles nili* also transmit the malaria (Yaya, 2011).

In Africa, members of *Anopheles gambiae* complex and *Anopheles funestus* are widely distributed and responsible for the transmission of malaria in the region. *Anopheles gambiae* also the most anthropophagic species in the complex and the most important probably the world‟s most efficient malaria vector with characteristic indoor and outdoor resting. *Anopheles arabiensis* and *Anopheles Quadriannulatus* are among the species of the *Anopheles gambiae* complex that are found in Ethiopia (Alemu *et al.,* 2011). *Anopheles arabiensis* occurs in most areas of tropical Africa and could be considered as a major target for control as a major vector where malaria transmission is stable. The highest transmission occurs with the beginning and end of the rainy season. *Plasmodium falciparum* is the main species and *Anopheles gambiae* is the main vector species in Ethiopia (Yaya, 2011).

Vector distribution of *Anopheles arabiensis* is the only species from the *Anopheles gambaie* complex known to be prevalent across malaria-endemic areas in Ethiopia. *Anopheles pharoensis* is a widely distributed *Anopheles* mosquito in the country and is considered to play a secondary role in malaria transmission along with *Anopheles funestus* and *Anopheles* nili. *Anopheles funestus* occurs frequently in localities along the swamps of the Baro and Awash rivers and the shores of Lake Tana in the north and the Rift Valley in the south; *Anopheles nili* can be an important vector in local transmission, particularly in the Gambella Regional State. The sporozoite infection rate of *Anopheles arabiensis* in Ethiopia ranges from a reported 0% to 5.4% (Yaya, 2011).

2.4. Severe *Plasmodium falciparum* malaria

Malaria is a mosquito-borne-infectious disease of humans and other animals caused by protozoan of the genus *Plasmodium*. Malaria is known to cause hemolytic anemia as major hematological disorder. Malaria usually presents with fever, chill with rigor, malaise, headache, myalgia, anorexia, vomiting, anemia and enlarged spleen. However, it can unusually present as coma, convulsion, bleeding manifestations, severe anemia, jaundice, black water fever, shock, hypoglycemia, metabolic acidosis and renal failure. It can rarely present as malarial hepatopathy, glomerulonephritis, nephrotic syndrome, syndrome and peripheral neuropathy (Yaya, 2011, Worku *et al*., 2014).

Severe malaria is most commonly caused by *Plasmodium falciparum*, although *Plasmodium vivax* and *Plasmodium knowlesi* can also cause severe malaria disease. Its complex life cycle depends on specialized protein expression within the host that determines the intra and/or extra-cellular parasite survival, the invasion of several types of cells and the ability to bypass the host‟s immune response (Gomes *et al*., 2011). The risk is increased if treatment of an uncomplicated attack of malaria caused by these parasites is delayed. Recognizing and promptly treating uncomplicated malaria is therefore of vital importance. Sometimes, however, severe *Plasmodium falciparum* malaria may develop so rapidly that early treatment of uncomplicated malaria is not feasible (Shafie and Eyasu, 2016).

Plasmodium falciparum causes severe complications as cerebral malaria, severe hemolytic anemia, acute renal failure, hypoglycemia and pulmonary infection. Almost all deaths from severe malaria result from infections with *Plasmodium falciparum* because severe malaria infections may cause vital organ dysfunction and destruction red blood cell lines. The two features that actually separate *Plasmodium falciparium* from the other human malaria have the ability to attack erythrocytes of all ages, causing high parasitaemia and enhanced growth and the capability to adhere to vascular endothelium through sequestration. *Plasmodium falciparium* is a threat to life because of high level of mortality and spreading drug resistance. Cerebral malaria caused by *Plasmodium falciparium* is when infected blood cells obstruct blood vessels in the brain; other vital organs can also be damaged often leading to death of patients (Gomes *et al*., 2011, Okwa, 2012).

Table 1:-Indicators of a worse severe malaria prognosis (Gomes *et al*., 2011).

Feature	Characteristics
World Health Organization 1990 criteria (WHO, 1990)	
Cerebral malaria	Coma not ascribed to other causes, Glasgow ≤ 9
Severe anemia	Hemoglobin < 7 g/dl or hematocrit < 20% with parasitemia >10,000/μl
Acute renal failure	Urinary output < 400 mL/24 hours in adult patients (< 12 mL/kg/24 hours in children) and serum creatinine > 3.0 mg/dL
Pulmonary edema	Radiographic changes and severe hypoxemia
Severe hypoglycemia	Blood glucose < 40 mg/dL
Shock	Systolic blood pressure < 70 mmHg in patients over 5 years of age (< 50 mmHg in children)
Abnormal bleeding and/or disseminated intravascular coagulation	Spontaneous nasal bleeding from the gastrointestinal tract or laboratory evidence of disseminated intravascular coagulation
Repeated generalized seizures	≥ 3 episodes within 24 hours
Metabolic acidosis	Arterial pH < 7.25 or HCO_3 < 15 mmol/L
Macroscopic hemoglobinuria	Non-secondary to glucose-6-phosphate deficiency hemolysis
Additional criteria published in 2000 (WHO, 2000)	
Consciousness status impairment	Altered consciousness level
Prostration or weakness	
Hyperparasitemia	>5% erythrocytes infected or > 250,000 parasites/μl in non-immune subjects
Hyperpyrexia	Body temperature > 40°C
Hyperbilirubinemia	Total bilirubin > 2.5 mg/dL

2.5. Hematological profiles and severe malaria

Changes in hematological parameters are likely to be influenced by any disease condition including endemic diseases such as malaria that can affects health of mankind with various clinical presentations (Akhtar *et al*., 2012, Singh *et al.*, 2014). Hematological changes are the most common complications in malaria and play major role in disease outcomes. These hematological abnormalities that have been reported to consistently companion which comprise anemia, thrombocytopenia and infrequently disseminated intravascular coagulation (Sumbele *et al*., 2016, Birhanu, 2017). Hematological changes that are associated with malaria infection include hemolytic anemia, thrombocytopenia and disseminated intravascular coagulation. Hematological alterations are some of the most common complications that cause changes in major cell lines such as red blood cells and platelets. Malaria affects almost all blood components and is a true hematological infectious disease. Anemia and thrombocytopenia are the most frequent malaria associated hematological complications and they play a major role in malaria pathogenesis, which have received more attention in the scientific literature due to their associated mortality (Kotepui *et al*., 2014, Sirak *et al*., 2016).

Anemia is a hematological disorder characterized by the reduction of the hemoglobin level to below the reference range depending on age, sex and altitude. Generally infection with *Plasmodium falciparum* produces significant changes of hematological parameters in severe malaria patients living in malaria endemic regions. Anemia is due to lytic destruction of red blood cells lines by the *Plasmodium falciparum* which is an intracellular parasite (Kosiyo, 2016). Malarial anemia (MA) is a multi-factorial disease for which the complex etiological basis is only partially defined. Severe malarial anemia is one of the main clinical presentations of severe malaria caused by *Plasmodium falciparum*. The causation of severe malaria anemia in malaria endemic areas may include a number of discrete as well as overlapping features, such as lysis of infected and uninfected RBCs spleen sequestration of RBCs, dyserythropoiesis and bone marrow suppression infectious diseases and chronic transmission of malaria. While hematological insults resulting in moderate and severe anemia in infection with *Plasmodium falciparum* have been established (Sumbele *et al.,* 2016)

The most commonly affected hematological parameters by the severe malaria are red blood cells, hemoglobin, hematocrit and platelets. Thrombocytopenia is a hematological disorder resulting in the reduction of the number of platelets in the peripheral blood to below the reference range. Thrombocytopenia is associated with parasite density and therefore may be a marker of disease severity. The normal platelet count is 150-400 x $10^3/\mu L$. It is a common and early sign of severe malarial infection (Agravat and Dhruva, 2010, Kosiyo, 2016). The reduced platelet counts observed in some severe malaria patients may be related in part to pseudo-thrombocytopenia but immune mediated thrombocytopenia may not be ruled out. Although there was a trend towards platelet aggregation being associated with thrombocytopenia but the frequency may still be primarily associated with malaria parasitaemia. Although *Plasmodium falciparum* infection is generally associated with a pro-coagulant status that is characterized by thrombocytopenia as well as coagulation cascade and fibrinolytic system activation, bleeding events are uncommon (Gomes *et al*., 2011).

Malaria is a major cause of deaths in the tropical and sub-tropical area of the world by hematological and biochemical changes of the most common complications in malaria and they play a major role in malaria pathogenesis. Malaria infected patients tended to have significantly lower platelets level in comparison to non-malaria infected patients. The most common complication during malaria infection is thrombocytopenia. Persons with platelet counts less than 150,000/µl were 12-15 times more likely to have malaria infection than persons with platelet counts greater than 150,000/µl (Kotepui *et al.,* 2014). Platelet abnormalities in malaria are both qualitative and quantitative change. It implies that thrombocytopenia may be a marker of *Plasmodium falciparum* infection (Leal-Santos *et al*., 2013).

Plasmodium falciparum is the main cause of malaria-related death worldwide; however, other species can also cause serious illness (Al-Salahy *et al*., 2016). As parasites of the blood for the majority of their complex life cycle, they expectedly induce hematological alterations. The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia and thrombocytopenia (Kumar *et al*., 2015). Patients with the highest parasite densities also have the highest fatality rates. High parasitemia due to *Plasmodium falciparum* infection takes a serious turn in anemia. Moreover, excessive hemolysis of parasitized RBCs in malaria infection may lead to anemia (Bashawri, 2002). Thrombocytopenia was also seen in the majority of patients with severe malaria. The incidence of thrombocytopenia was more common among patients suffering from complicated *Plasmodium falciparum* malaria. The highest thrombocytopenia occurs at 5-6 days after infection and becomes normal five to seven days after parasite clearance. During malarial infection, despite the presence of thrombocytopenia, the number of megakaryocytes in the bone marrow remained adequate or increased (World Health Organization, Geneva, Switzerland, 2008).

Hematological abnormalities are considered a hallmark of severe malaria and are reported to be most pronounced in *Plasmodium falciparum* infections because of *Plasmodium falciparum* malaria is one of the most common causes of anemia. Anemia and thrombocytopenia are the most frequently found abnormality in patients suffering with severe malaria (Malik *et al.,* 2010, Singh et al., 2014). The severity of hematologic disease caused by *Plasmodium falciparum* is related to the ability of the parasites to invade and grow in different red cell populations as well as the intrinsic growth rate of the parasite. Anemia is consequently an inevitable outcome of malaria infection, but the hematocrit in some patients with high parasite densities does not decrease as much as would be expected from the destruction of the parasitized red blood cells. Similar to hemoglobin levels and hematocrit values vary according to age, gender and altitude (Agbenyega *et al*., 2000, Kimbi *et al*., 2013).

2.6. Statement of the problem

Severe malaria is a major public health challenge for many countries in the world causing tremendous rates of morbidity and mortality (Jennison *et al*., 2015). The *Plasmodium falciparum* species infections are responsible for the majority of the human severe malaria burden worldwide. This species is found worldwide in tropical and sub-tropical regions. It is estimated that every year approximately one million people are killed by species, especially in Africa where this species predominates. The burden of malaria is increasing, especially in sub-Saharan Africa, because of drug and insecticide resistance multiples rapidly in the blood and can thus cause abundant blood loss and social and environmental changes. When this occurs in the brain, cerebral malaria results a complication that can be fatal. In addition, the infected parasites can clog small blood vessels (Greenwood and Mutabingwa, 2002, Abdel-gadir, 2015).

Malaria is commonly associated with various degrees of hematological complications like anemia and thrombocytopenia. Hematological and biochemical abnormalities are considered a hallmark of malaria and statistical analyses have shown that many of these hematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt specific therapy even in the absence of a positive smear report for malaria. Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications. The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, and rarely Disseminated Intravascular Coagulation (DIC).

Thrombocytopenia may be associated with bleeding tendency which is one of the important severe manifestations of *Plasmodium falciparum* malaria (Kidist, 2015).

Malaria also remains the leading public health problem in Ethiopia. The problem covers 75% of the landmass of the country and 68% of the population are living in malaria-risk areas. According to Ethiopian Federal Ministry of Health malarias are defined as being located below 2000m altitude and it is responsible for 17% of outpatient visits 15% of hospital admissions and 29% of inpatient deaths. Approximately 70,000 people die due to severe malaria each year in Ethiopia. Nationally the prevalence of malaria was 1.3 % in areas below 2000m with 77% of the positive tests showing *Plasmodium falciparum* infections (Yaya, 2011, Abeku *et al.,* 2015). September to December following the main rainy season from June to August is highly transmission period. The transmissions are common and the distribution varies from place to place depending on climate and altitude (Deressa, 2004). It is limited by the lack of water collections for mosquito breeding and low humidity due to low rainfall and sparse vegetation. Despite the current efforts to control malaria in Ethiopia, the situation has not improved mainly due to the increasing problems of parasite resistance to the relatively cheaper anti-malarial drugs, vector resistance to insecticides, low coverage of malaria preventive services, poor access to health care, rudimentary health service infrastructure, large population movements and limited financial and human resources (President Malaria Intiative Operational Plan Ethiopia FY, 2015)

Gambella Region is characterized by high malaria transmission due to its suitable climate, humidity, hot and high rainfall. *Plasmodium falciparum* and *Plasmodium vivax* contribute to most of the malaria cases of the general population and *Anopheles gambiae* and *Anopheles pharoensis* are the primary mosquito vector species. *Plasmodium falciparum* is the predominant species in Gambella where it is responsible for more than 90% of the malaria cases, with only minimal cases caused by *Plasmodium vivax.* Studies on fasting blood glucose levels and blood hematological parameters of *Plasmodium falciparum* infected patients had to be undertaken in highly infested regions of Ethiopia. Therefore, the present research aimed to study the above parameters in Gambella Region at Gambella General Hospital, South Western Ethiopia. The deterioration of malaria situation in Gambella Region is due to appearance of parasites resistant to anti-malarial drugs and vector resistance to some insecticides in use. Other contributing factors are inadequate financial resources, poor community participation and the lack of expert staff due to brain drain. These are coupled with poor health coverage and weak information system in a large country like Ethiopia. The Gambella regional Health office also considers malaria a national priority and remains firmly committed to malaria control, diagnosis and treatment in Gambella region. This is in line with the Gambella regional Health office of a region free of preventable diseases and ill health and the regional development agenda as outlined in Gambella vision 2030, and the aims of Millennium Development Goals (Gambella Regional Health Bureau report, 2017).

Worldwide, there is broad variation in hematological patterns among different population groups. Therefore, evaluation and monitoring of modifiable risk factors can be beneficial to reduce severe malaria morbidity and mortality of the patients. The present study will evaluate and examine fasting blood glucose and hematological parameters and other associated risk factors in severe malaria in the study area and give recommendations so as to minimize severe malaria morbidities and mortalities.

2.7. Rationale and significance of the study

The crucial value of this investigation was to look for the biochemical and hematological parameter changes in patients infected with severe malarial parasites of *Plasmodium falciparum*. Malaria is one of the dominant health problems of Ethiopia that attacks the working peasant population, which amounts about 85% of the population. For this purpose one of the severe malaria infested region of Ethiopia, Gambella region was chosen and the patients from OPD admitted in the Gambella General hospital were recruited for investigations.

Changes in biochemical and hematological parameteres values are frequent in malaria infected individuals irrespective of sex and anemic conditions and reported from different parts of Ethiopia. The present study was conducted in Gambella region at Gambella General Hospital of South Western Ethiopia through screening patients for the prevalence of anemia, thrombocytopenia and hypoglycemia by looking into the various biochemical and hematological parameters such as RBC, PLT, Hb, Hct, MCV, MCH, MCHC and fasting blood glucose from the whole blood samples. It is also hoped that a significant number of patients were screened for parasitemia and controlling the insect vectors of *Plasmodium falciparum* was recommended for the severe malaria patients.

Anemia and thrombocytopenia may also hint at severe malaria infection and platelet count may be major clinical value to be requested by the clinicians. Microscopic diagnosis is the perfect gold standard for malaria parasite detection and speciation. This technique requires technical expertise and is time-consuming for repeated smear examinations. However, it is a valuable technique when performed correctly in the right hands but can be unreliable and perceived as wasteful when poorly executed. The information gained from this study may be helpful to healthcare providers, especially the clinicians in implementing an empirical differential diagnosis of malaria. Therefore, evaluation and examination of biochemical parameters and hematological profiles and other associated risk factors in patients with severe malaria in our setup was significantly important to help shape clinical as well as public health care of the patients and the population. In addition, the results obtained from this study are expected to pave the way for further related studies to be broadly and extensively performed.

2.8. Hypothesis of the study

- Inter-individual variation exists in biochemical parameters (fasting blood glucose) and hematological parameters (RBC, Hb, Hct, MCV, MCH, MCHC and PLT) levels in patients with severe malaria caused by the *Plasmodium falciparum*.

- Inter-individual variation does not exist in biochemical parameter (fasting blood glucose) and hematological parameters (RBC, Hb, Hct, MCV, MCH, MCHC and PLT) levels in patients with severe malaria caused by the *Plasmodium falciparum.*

3. OBJECTIVES

3.1. General objective

- To compare the changes in biochemical parameters and hematological parameters among male patients infected with severe *Plasmodium falciparum* malaria and healthy individuals in Gambella Region, of South Western Ethiopia.

3.2. Specific objectives

- To measure the changes in fasting blood glucose profiles in patients with severe malaria caused by *Plasmodium falciparum* and control groups among adult males.
- To determine severity of malaria through microscopy measurement in adult males.
- To quantify hematological parameters (RBC, Hb, Hct, MCH, MCV, MCHC and PLT) of severe malaria caused by *Plasmodium falciparum* among adult males.
- To assess the presence and severity of anemia and thrombocytopenia in severe malaria patients caused by *Plasmodium falciparum*.

4. MATERIALS AND METHODS

4.1. Study design and period

This is a cross-sectional comparative study based on the clinical and laboratory data of patients with severe malaria caused by *Plasmodium falciparum* and control groups who attended at the General Hospital of the Gambella region between November, 2016 and February, 2017.

A total of 100 study subjects i.e. 40 healthy male (as a control) and 60 severe malaria infected adult males were enrolled in the study. Thick blood film using anti-coagulated (EDTA) venous blood was employed in the detection of malaria parasite. Four milliliters of blood sample was drained from study subjects by a trained laboratory technologist after screening first for *Plasmodium falciparum* by methods routinely used for parasitological confirmation microscopically. The study was conducted between November, 2016 to February, 2017. The study was carried out on confirmed malaria positive patients of *Plasmodium faliciparm* attending the OPD of Gambella General Hospital.

Hematological parameters were measured as a part of the routine complete blood count (CBC) test from a blood sample and fasting blood glucose from the whole serum. Several methods exist for measuring hematological parameters most of which were done currently by automated machines (auto hematological analyzer) designed to perform different tests on blood and fasting blood glucose was measured by using human analyzer machine.

4.2. Description of the study area

Gambella General Hospital which serves an estimated 20,000 patients is the only hospital in the Region. The region has an area of 25,800 km^2 with an estimated total population of 92,474 having six major native Nilotic ethnic groups, namely, *Anuak* (also called *Anywaa*), *Nuer*, *Majenger*, *Opo*, *Surma* and *Komo*. The *Nuer* and the *Anuak* are the dominant groups in the region and in Gambella town settlers of various ethnic origins who moved from all over Ethiopia are loosely defined as “highlanders” also dwell in the region as minorities.

Gambella is one of nine regional states of Ethiopia, located approximately 776 km South West of Addis Ababa. Most of the region is flat, hot, and humid, with an altitude range of 300–2,000 m above sea level and sloping westward.

Gambella town is located in a lowland area with average altitude of 300–500 metres above sea level. The main rainy season of the region is between May and October and from November through April it is mostly dry. According to this same source the mean annual temperature of the region ranges from 24.3 to 31.3°C and the annual monthly temperature vary between 27 and 43°C throughout the year. The absolute maximum and minimum temperatures are 45.0 and 21.3°C occurring in mid of March, February and December. The average yearly rainfall is 1200 mm. Subsistence cultivation of maize and sorghum along the sides of the major rivers Baro, Akobo and Gilo is practiced by the *Anuak*. Fishing and forest hunting are supplementary income sources. Most *Nuers* who mainly live in the driest Ethio-Sudanese border of the region largely depend on livestock.

4.3. Population

4.3.1. Source population

The source population for this study was all malaria patients attending at Gambella General Hospital.

4.3.2. Target population

All male individuals of age group from 18-55 years attending in Gambella region attending the OPD of Gambella General Hospital.

4.3.3. Study population

All adult males individuals (aged from 18-55 years) microscopically confirmed as having *Plasmodium faliciprum* mono-infection at Gambella General Hospital.

4.4. Inclusion and Exclusion criteria

4.4.1. Inclusion criteria

All adult male with febrile between18-55 years of age, who permanently reside in the study area and who are positive for *Plasmodium faliciprum* with illness of at least 3-5 days.

- ✓ Only individuals who volunteer to participate by giving informed consent.
- ✓ Patients positive only for *Plasmodium falciparum* malaria parasite by peripheral smear.

4.4.2. Exclusion criteria

- ✓ Patients having other co-infections like enteric fever, diabetic mellitus, hypertension etc.
- ✓ Severe malaria patients caused by *Plasmodium falciparum* who are under medication.
- ✓ Patients having mixed malarial infection.
- ✓ *Plasmodium falciparum* positive and having illness of more than five days and obese adult males (BMI>27).

4.5. Sampling technique

Blood samples were collected from patient into EDTA containers for the thick blood film preparation to detect the presence or absence of malaria parasites and determine full blood counts (FBCs) involving, RBC, Hb, Hct, MCV, MCH, MCHC and PLT count or thrombocyte count. Thick blood films were stained by the field staining technique to confirm infection with

Plasmodium falciparum malarial parasite by microscopic examination of Giemsa stained thick blood smears.

A probable purposive sampling technique was implemented to select the Gambella General Hospital. The purpose and practical consequences of participating in this study was explained to the participant.

4.6. Sample size determination

The sample size was determined based on prevalence of malaria (19.6%) in Ethiopia as reported by systematic meta-analysis (Sirak et al., 2016)**.** The number of study subjects selected to represent the study population was calculated using the statistical formula shown below . It mportant to make inferences based on the findings from the sample. Sample size was determined by using single population formula 95 % CI and 5% marginal sample error assumed to be = 0.05, Z = 1.96.

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

Where Z, P, d, n, and α represents

$Z_{1-\alpha/2}$ = the standard normal variable at (1-α) % confidence level and α (level of significance) is mostly taken to be 5%.

- Usually 95% confidence level is used Z = 1.96

d = the margin of sampling error tolerated.

P = Estimate of the prevalence rate severe malaria patients in the population and

n = Minimum sample size required for very large population (≥ 10,000).

After adjustment Based on the formula sample size (N = 196) but due to budget constraint only 100 were enrolled and 60 microscopically *Plasmodium faliciparum* postive(*Pf*$^{+4}$) or using microscpe test individuals with severe malaria and 40 controls which have no microscopic *Plasmodium faliciparum* in their blood.

4.7. Study variables

4.7.1. Dependant variables

- ✓ Hemoglobin
- ✓ Red blood cells
- ✓ Hematocrit (Relative Volume of Erythrocytes)
- ✓ Mean Corpuscular (erythrocyte) Volume
- ✓ Mean Corpuscular (Erythrocyte) Hemoglobin
- ✓ Mean Corpuscular (Erythrocyte) Hemoglobin Concentration
- ✓ Platelet
- ✓ Fasting blood glucose

4.7.2. Independent variables

- ✓ Socio-demographic factors
- ✓ Anthropometric indicators
- ✓ Proportion of individuals infectiousness of severe malaria parasite density (PF^{+4})

4.8. Ethical Approval

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/DRERC/BCHM031/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Collaboration letter for data collection was also obtained from Gambella General Hospital. Written Informed consent for study participation (for the collection of blood samples) was obtained from each individual who were willing to participate in the study. All information provided was remain confidential and would only be reported as group data with no identifying information. All data, including questionnaires was kept in a secure location and only those directly involved with the research have access to them. After the research is completed, excess samples were discarded and the questionnaires also destroyed.

4.9. Investigation of blood samples

Venous blood was collected from each subject by a qualified laboratory into EDTA precoated tubes and the age, sex and number of the subjects marked carefully on the tubes using masking tape. Slides were also labeled and the number of the subjects marked on them. Thin blood films were smeared on clean dry slides for malaria parasite identification. They were light-dried before being packed in slide racks.

For malaria parasitaemia quantification the slides were stained with 10% Giemsa solution stain and examined under the light microscope using oil immersion objectives (100X) (WHO, 2009). The intensity of parasitaemia was measured per high power field or microscopic field which the gold standard and preferred option for diagnosing malaria and this gold standard for detecting malaria and identifying the species of *Plasmodium* involved. In nearly all cases, examination of thick and thin blood films was reveal malaria parasites. Thick films are more sensitive than thin films for detecting low density malaria parasitaemia. Up to 5-10 high power fields were examined before intensity was confirmed and the number of parasites per field noted. Severity of parasitaemia was assessed as parasitic index (P1) and expressed as scanty, mild and severe (++, +++ and ++++ i.e PF^{+2}, PF^{+3} and PF^{+4}) (WHO, 2009).

After checking for severity of malaria the four ml blood sample was used for measurement of hematological parameters such as RBC, hemoglobin (Hb), hematocrit (Hct), MCV, MCH, MCHC and PLT using auto sampler CBC machine (Automated CBC Analyzer: Sysmex KX-21) and the blood was transferred to a two milliliters vacutainer tube then fasting blood glucose was measure using human analyzer after centrifuge.

4.10. Collection of blood samples

A total of 100 blood samples i.e. 40 healthy males and 60 malaria adult males severe patients were screened for presence of *Plasmodium falciparum*. 4 mls of venous blood was collected from adult males at the OPD or parasitology laboratory and their names, age and gender other demographic informations were recorded. Four milliliters of blood was poured in to EDTA Vaccutainer tube and properly labeled. Malaria infection was diagnosed by microscopy which was the gold standard and preferred option for diagnosing malaria. A thick and thin smear was prepared. Thick smears were dehaemoglobinized and stained with Giemsa-stained and focused under 100x oil emersion lens.

All valuable demographic data were obtained through questionnaire prepared for this purpose which shall be filled by willing participants or their guardians or data collectors may help filling the same. Clinical data were obtained from the patient card through trained health professionals. All laboratory based results was recorded in a log book for further analysis

4.11. Malaria diagnosis by microscopy

Blood samples were collected for thick blood film microscopic examination or rapid diagnostic test. The blood film was stained as follows: first finger prick blood samples was dropped on the testing and smeared as thin and thick films which is considered the gold standard in malaria diagnosis. Parasitemia can be quantified, which may assist in both the prediction of *Plasmodium falciparum* infection severity and in the therapeutic monitoring of the infection and allowed to air dry or light-dry then the thick blood smear was fixed with absolute methanol carefully but not to fix the thin smear. Then the slides was immersed in to 10% Giemsa stained solution which recommended for identification of *Plasmodium* species and for parasite count for 15 minutes and briefly rinsed with tap water to wash the stain and air dried to confirm the presence of *Plasmodium falciparum* parasites in addition to clinical diagnosis of malaria. Finally the Giemsa stained blood films was observed under light microscope with 1000X magnification by using oil immersion and the parasite density was determined by the amount of relative parasite count in positive smears using a simple code from one to four crosses (+ to ++++ or PF^{+1} to PF^{+4}) according to Brace-chwat. For levels of malarial parasitemia data were grouped into high parasitemia (>10 parasite/ 1 oil field), moderate parasitemia (1–10 parasite/ 1 oil field) and low parasitemia (1–100 parasite/100 oil field) groups (Lewis, 2006).

4.12. Parasitological examination

Malaria darasite density of *Plasmodium falciparum* was determined in various blood smears stained by Giemsa stain. The presence and relative parasite count of *Plasmodium falciparum* in each blood sample was determined from Giemsa stained thin and thick films after staining for 15 min. The identification of the species of human parasites in the blood films was carried out according to WHO method (WHO, 2009). A slide was scored as negative when 100 high power fields (at 1000x magnification) had been examined for about 30 min without seeing any parasites.

- ✓ + (PF^{+1}): 1-10 parasites per 100 thick film fields.
- ✓ ++ (PF^{+2}): 11-100 parasites per 100 thick film fields.
- ✓ +++ (PF^{+3}): 1-10 parasites per one thick film fields.
- ✓ ++++ (PF^{+4}): More than 10 parasites per one thick film field or > 10 parasites/field examined

From those different level of parasitaemia ++++ (PF^{+4}) more than 10 parasites per one thick film field or > 10 parasites/field examined were taken for this study.

4.13. Hematological and Biochemical Analysis

Venous blood in male adults was obtained from the median cubital vein as per established Standard Operating Procedures (SOPs). The blood was transferred to a four mililiters EDTA vacutainer tube and mixed thoroughly to prevent clotting. Whole blood samples were collected in EDTA tube for determination of hematological parameters including red blood cells (RBC), hemoglobin (Hb) concentration, hematocrit (Hct), MCV, MCH, MCHC and PLT using automated (SYSMX.KX-21n) hematology analyzer. MCH also can calculated from hemoglobin level and RBC count. In addition, MCHC was calculated according to the hemoglobin and hematocrit values. The hematocrit was measured as a function of the numeric integration of MCV (Carberand *et al*., 1999).

$$MCV = \frac{Hematocrit}{RBC\ count}$$

$$MCH = \frac{Hemoglobn}{RBC\ count}$$

$$MCHC = \frac{Hemoglobin}{Hematocrit}$$

4.14. Anthropometrical measurement procedure

The weight of the severe of *Plasmodium falciparum* malaria and the control groups was measured using a standard balance and the height was measured by using a height measuring device attached to the balance. Body Mass Index (BMI) was then calculated from the body weight (kg) and height (meter) as follows:

BMI = Weight (in kg) / (Height in meter) ^{2} (Tambe *et al*., 2010). Using the WHO classification four categories of BMI can be identified as follows:

- Underweight <18.5 kg/m2
- Normal 18.5–24.9 kg/m2
- Overweight 25.0–29.9 kg/m2 and
- Obesity >30 kg/m2.

The participants‟ ages were also recorded. To determine the anemia, thrombocytopenia and hypoglycemia status of the severe malaria patient‟s height, weight and age measurements were taken for patients and compared with the standard recommended scale.

4.15. Data quality control and management

- The data collection questionnaire was well prepared and all variables were filled on the data extraction format daily.
- All the laboratory procedures were handled by professional laboratory technologists.
- All the tests were standardized and automated.

4.16. Data processing and statistical analysis

Laboratory analyses of the blood samples and questionnaires was performed by coding and entering the data into a computer and analyzed using Epidata statistical software version 3.1 and then exporting the entered data to SPSS software version 23 package the different variables were tested and analyzed. The data were expressed as mean ± SD and percentages. The results were analyzed statistically using column statistics and descriptive statistics. Correlation among the investigated parameters and demographic data were tested. Differences proportions were tested by descriptive statistics frequency tests and student"s t tests for independence as appropriate. An independent *t*-test was used to show mean differences of hematological biochemical profiles among severe malaria patients and control groups.

Multiple linear correlation analysis was used to show whether there was significant correlation between severe malaria infection and control groups of hematological parameters, fasting blood glucose and anthropometric parameters. Descriptive statistical tests were also used for analysis of demographic and hematological data. Association between variables was evaluated using Pearson correlation test. In all analysis, significance level was considered at 95% confidence interval ($P < 0.05$).

5. RESULTS

A total of 100 male adults were recruited for the study. Sixty adult males with severe *Plasmodium falciparum* malaria had high intensity of infection PF^{4+} (>10/one field) while the remaining were negative and were used as control groups. The most common form of complicated severe malaria among the studied 60 patients in Gambella region at Gambella General Hospital was cerebral malaria followed by prostration and weakness as a patient who could not sit or walk with no obvious neurological explanations. All of the study participants were males for cases and controls with high parasitemia. The mean age of the study participants was 28.20±9.133 and 29.50±9.143 years for the severe malaria patients and control groups respectively with predominant age group was 19–35 years ranging from 18 to 55 years (Table 2). There was a good matching with regards to age, weight and height between the control and study groups. The demographic data of the two groups is shown in Table 2 below. There were significant decreases in the mean values of the fasting blood glucose (FBG), red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct) and platelets (PLT) in patients with severe *Plasmodium falciparum* malaria in comparison to healthy subjects as shown in Table 6.

5.1. Anthropometric and clinical features of the severe malaria patients

Anthropometric measurements were performed to see the prevalence of change in biochemical parameters between control groups and study subjects by measuring the weight, height, body mass index of patients. The present study revealed that the mean body mass index is almost in a normal range (20.85-21.50 Kg/m2) in this study participant. Almost 98% of the severe malaria patients and the control groups were normal. The mean± standard deviations for the body mass index severe malaria patients and in control groups of were 20.85±2.161 and 21.50±2.562 respectively from Table 2.

There was no significant reduction in the age, height, weight and body mass index in adult males with severe malaria patients compared to the control groups ($P > 0.05$). Height level in the subjects with severe malaria was almost the same to the control groups 1.7070±0.6715 and

1.7160±0.6644 respectively (P > 0.05). The mean weight was also the same in subjects with severe malaria patients when compared with the control groups 61.20±6.864 and 63.18±6.296 (P > 0.05). Therefore, in this finding *Plasmodium falciparum* infection of severe malaria does not altered the anthropometric parameters as compared with the control groups.

Table 2:- Comparison of mean anthropometric and biochemical parameters measurement of severe malaria patients and control groups of adult males at Gambella General Hospital, Ethiopia, November, 2016 to February, 2017.

Variables	**Severe malaria patients (N=60) Mean ± SD**	**Control Group (N=40) Mean ± SD**	**P-value**
Age	28.20±9.133	29.50±9.143	0.488
Weight	61.20±6.864	63.18±6.296	0.142
Height	1.7070±0.6715	1.7160±0.6644	0.577
BMI	20.85±2.161	21.50 ±2.562	0.191

The variables are expressed as mean ± standard deviation and **p-value highly significant at the 0.01 level.

5.2. Levels of biochemical parameters in the severe malaria patients

The fasting blood glucose level was measured for controls as well as for the severe malaria patients and the results showed that there was a significant difference between the two groups. The severe malaria patients had lower mean average fasting blood glucose level (91.60± 13.431 mg/dl) than the control groups (111.05 ± 10.208 mg/dl) with a p-value < 0.01 as shown from Table 2. The mean values represented 11.2% drop in blood fasting blood glucose concentrations compared to their corresponding control groups. Therefore, the fasting blood glucose level had really gone down for the severe malaria patients than control groups may be due severe of *Plasmodium falciparum* malaria. Mean fasting blood glucose level of severe malaria patients was 91.60 mg/dl with standard deviation of 13.431 mg/dl, whereas, mean fasting blood glucose level of control groups was 111.05 mg/dl with standard deviation of 10.208 mg/dl. This difference was statistically significant (p-Value < 0.01).

Biochemical and hematological indicator parameters of severe malaria such as fasting blood glucose, red blood cells, hemoglobin, hematocrit and platelets were used to evaluate the prevalence and to categorize control groups and study groups into severe, moderate, mild and normal of anemia, thrombocytopenia, hypoglycemia and hyperglycemia. Cut-off point values of < 7g/dl for hemoglobin indicated severe anemia due to severe malaria of *Plasmodium falciparum*, 7–9.9 g/dl moderate risk of anemia and > 12.9g/dl was considered as normal. Based on this cut- off point, values obtained for the study group and control group were assessed and compared. Among the severe malaria adult males patient 33(55%) were mildly anemic, 11 (18.3 %) were moderately anemic and 16 (26.7%) were normal. Additionally 31 (77.5 %) of control groups were categorized as normal value as shown in Table 3.

In order to assess the prevalence of anemia with cut-off point of 36-45% hematocrit, as normal and < 21% hematocrit as severe anemia. Based on the cut-off point it was found that 47 (47%) was considered normal, 31(77.5 %) from control groups and 16 (26.7%) was from severe anemia adult male group, there were no severe anemia in adult males from severe malaria as well as from control groups. Both hematological laboratory indicators such as hemoglobin and hematocrit with certainty showed significant change due to severity of malaria (p < 0.01) as illustrated in Table 3.

With regard to anthropometric indicators of severe malaria patients and control groups, body mass index was used to categorize individuals into underweight, normal, over weight and obese. Hence the result showed that the severe malarious patients 5 (8.3%) were underweight, 51(85%) were normal, 4 (6.7%) were overweight and none were obese and for the control groups 4(10%) were under weight, 32(80%), overweight, 4(10%) were normal weight and none were obese as shown in Table 3.

Table 3:- Categorization of degree of severity anemia, thrombocytopenia and level of glucose between study and control groups by using biochemical profiles and hematological parameters at Gambella General Hospital, Ethiopia, November, 2016 to February, 2017.

Variables	Severe malaria (60) N (%)	Control groups (40) N (%)	Total (100) N (%)	p-value
BMI(kg/m^2)				0.191
Under Weight	5(8.3)	4(10)	9 (9)	
Normal Weight	51(85)	32(80)	83 (82)	
Over Weight	4(6.7)	4(10)	8(9)	
Obese	0(0)	0(0)	0(0)	
RBCx10^6 cells/µl				0.000**
Severe anemia	0(0)	0(0)	0(0)	
Moderately anemia	8(13.33)	3(7.5)	11(11)	
Mild anemia	27(45)	13(32.5)	40(40)	
Normal	25(41.66)	24(60)	49(49)	
Hb(g/dl)				0.000**
Severe anemia	0(0)	0(0)	0(0)	
Moderately anemia	11(18.3)	1(2.5)	12(12)	
Mild anemia	33(55)	8(20)	41(41)	
Normal	16(26.7)	31(77.5)	47(56)	
Hct(%)				0.000**
Severely anemia	0(0)	0(0)	0(0)	
Moderate anemia	11(18.3)	0(0)	11(11)	
Mild anemia	33(55)	9(22.5)	42(42)	
Normal	16(26.7)	31(77.5)	47(47)	
PLTx10^3/µl				0.000**
Severe Thrombocytopenia	0(0)	0(0)	0(0)	
Moderate thrombocytopenia	42(70)	1(2.5)	43(43)	
Mild Thrombocytopenia	17(30)	31(77.5)	49(49)	
Normal	1(1)	8(20)	9(8)	
FBG(mg/dl)				0.000**
Hyperglycemia	0(0)	1(2.5)	1(1)	
Normal	57(95)	39(97.5)	96(96)	
Hypoglycemia	3(5)	0(0)	3(3)	

Data are expressed as percentage and in numbers; **P value is statistically highly significant at 0.01 levels.

5.3. Levels of hematological parameters in the severe malaria patients

The average levels of the hematological parameters are shown in Table 4. The study showed statistically highly significant variation in RBC, Hb, Hct and PLT levels between the severe malaria patients and control groups ($p < 0.01$). However, the study showed no statistically significant variation between severe malaria patients and control groups and in their respective MCV, MCH and MCHC levels ($p > 0.05$) as shown in Table 4. In addition, independent samples t-test showed that the control groups had significantly higher mean levels of red blood cells, hemoglobin, hematocrit and platelets ($p < 0.01$) than all severe malaria patients. The average levels of MCV, MCH and MCHC were not changed and not statistically significant ($p > 0.05$). Generally RBC, Hb and Hct were significantly reduced in severe malaria patients compared to those with control groups ($p = 0.000$). Platelet count also was notably reduced in the severe malaria patients with high parasitemia compared to control groups ($p = 0.000$) (Table 4).

The hematological values in severe malaria-infected and control subjects are summarized in Table 4. There were significantly lower mean values of red blood cells, hematocrit, hemoglobin concentration and platelet count of 4.42 ± 0.787, 35.70 ± 5.509, 11.78 ± 1.878 and (138 ± 56.573) in severe malaria-infected adult males compared to 5.73 ± 1.062, 44.85 ± 3.468, 14.95 ± 1.176 and 249.95 ± 61.836 respectively in the control subjects ($P < 0.01$). Whereas MCV, MCH and MCHC there were no significantly lower mean values of 89.00 ± 7.045, 28.77 ± 2.769 and 32.77 ± 1.533 respectively in severe malaria-infected adult males compared to the control subjects 90.83 ± 14.564, 29.555 ± 1.987 and 33.23 ± 2.293 respectively with ($p > 0.05$).

From Table 4, the mean value of Hb in patients with severe *Plasmodium falciparum* malaria (11.78 g/dl) was significantly lower than control groups (14.925 g/dl) with (P value = 0.000), similarly the mean value of RBC in patients with *Plasmodium falciparum* severe malaria (4.42 ± 0.787) also significantly lower than control groups (5.73 ± 1.062) with (P value = 0.000). The mean of PLT count was significantly lower in patients with *Plasmodium falciparum* severe malaria ($138.27 \times 10^3/\mu l$) than control groups ($249.95 \times 10^3/\mu l$ (P value = 0.000). Generally from Table 4 mean value of red blood cells, hemoglobin, hematocrit and platelets were lower in patients with severe malaria of *Plasmodium falciparum* compared to those control groups.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were did not show significant statistical differences in relation to the type of complications in severe malaria ($p > 0.05$) as shown in Table 4. There was no significant association between severe malaria and control groups with reference to MCV, MCH and MCHC cutoff value (P values > 0.05).

Table 4:- Concentrations (mean±SD) of fasting blood glucose (FBG), RBC, Hb, Hct, MCV, MCH, MCHC and PLT in severe malaria patients and control groups of adult males at Gambella General Hospital, Ethiopia, November, 2016 to February, 2017.

Variables	Severe malaria patients (N=60)	Control Group (N=40)	P-value
FBG[a]	91.60±13.431	111.05±10.208	0.000**
RBC[b]	4.42±0.787	5.73±1.062	0.000**
Hb[c]	11.78±1.878	14.95±1.176	0.000**
Hct[d]	35.70 ±5.509	44.85±3.468	0.000**
MCV[e]	89.00±7.045	90.83±14.564	0.259
MCH[f]	28.87±2.769	29.555±1.987	0.105
MCHC[g]	32.77±1.533	33.23±2.293	0.271
PLT[h]	138.27±56.573	249.95±61.836	0.000**

Data are expressed as Mean ±SD; *P- value ≤0.05 is statistically significant and **p-value is highly significant at 0.01 level. Values bearing different superscripts [a, b, c, d, e, f, g, h] represent units, [a]milligram per deciliter, [b]10^6 cells/μl, [c]gram per deciliter, [d]percent, [e]femtoliter; [f]picogram;; [g]gram per deciliter and [h]10^3 x cells/μl.

5.4. Association between anemia and severe malaria

Defining anemia: The WHO defines anemia as:

- ✓ Severe anemia: Hb < 7.0 g/dl or Hct < 21%
- ✓ Moderate anemia: Hb, 7.0 – 9.9 g/dl or Hct, 21%-29.7%
- ✓ Mild anemia: Hb, 10.0 – 12.9g/dl or Hct ,30%-38.7%
- ✓ Normal: Hb > 12.9 or Hct > 38.7%

According to the WHO cutoff value for anemia was defined as Hb level < 12.9g/dl and for the hematocrit level < 38.7% for adult males and Hb level < 7g/dl and for the hematocrit < 21% were severe anemia. Therefore, the result show in Table 5, (55%) were mildly anemia from the 60 severe malaria patients of (26.7%) of them were normal whereas (18.33%) of them were moderate anemia‟s but no severe anemia. Anemia also defined as by the value of the red blood cells counts less than $4.5x10^6$ cells/µl. Therefore according to Table 5, 45% were mildly anemia, 13.33% were moderately anemic, 41.66% were normal and there was no severe anemia in the severe malaria patients infected with *Plasmodium falciparum*.

Table 5:- Prevalence of anemia assessed by different methods in 60 severe malaria adult male patients at Gambella General Hospital, Ethiopia, November, 2016 to February, 2017.

Variables	Normal %	Severe anemia %	Moderate anemia %	Mild anemia %
RBCx10^6 cells/µl	41.66	0	13.33	45
Hb (g/dl)	**26.7**	**0**	**18.33**	**55**
Hct(%)	25.7	0	19.33	55

Data are expressed as percentage.

5.5. Association between thrombocytopenia and severe malaria

Thrombocytopenia is defined as platelet count < $150 \times 10^3 / \mu l$ and further defined as severe if the platelet count is < $50 \times 10^3 / \mu l$.

- Thrombocytopenia was classified as
  - ✓ Severe (PLT: < $50x10^3/\mu l$).
  - ✓ Moderate (PLT: $50x10^3/\mu l$ to $75x10^3/ \mu l$)
  - ✓ Mild (PLT: $75x10^3/ \mu l^3$ to $150x10^3/ \mu l$) and
  - ✓ Normal (PLT: $150x10^3/ \mu l$ - $400x10^3/ \mu l$

Thrombocytopenia was defined as platelet count < $150 \times 10^3/ \mu l$ and further defined as severe if the platelet count is < $50 \times 10^3/\mu l$. The mean platelet count for the severe malaria infected group was significantly lower than control groups ($138.27 \times 10^3/ \mu l$ Vs $249.95 \times 10^3/ \mu l$); ($p = 0.000$) (Table 6). Mild thrombocytopenia was observed in 63.3% (n = 38), 5% (n = 3) moderate thrombocytopenia, 31.7% (n = 19) were normal and no severe thrombocytopenia seen in severe malaria infected patients. Additionally mild thrombocytopenia 2.5% (n = 1) and no severe and moderate thrombocytopenia were seen in the control groups as shown from Table 6.

Table 6:- Distribution and prevalence of platelet counts in severe malaria patients and control groups at Gambella General Hospital, Ethiopia, November, 2016 to February 2017.

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Platelet count	Degree of Thrombocytopenia	Number of severe malaria (%) N=60	Number of control groups (%) N=40
<$50x10^3/\mu l^3$	Severe	0(0%)	0(0%)
50 -75 × $10^3/\mu l$	Moderate	3 (5%)	0(0%)
75 -150×$10^3/\mu l$	Mild	38 (63.3%)	1 (2.5%)
150 -400 × $10^3/\mu l$	Normal	19(31.7%)	39 (97.5%)

Data are expressed as percentage;

5.6. Socio-demographic and Anthropometric characteristics of the dependent variables

Bivariate Pearson correlation analyses for different anthropometric, biochemical and hematological measurements for the severe malaria patients and control groups were done and results are shown in Table 7. Hemoglobin significant positively correlated with demographic indicators ($r = 0.108$, $p = 0.0410$) age in study groups. However, there is non-significant negative correlation with anthropometric in body mass index ($r = -0.052$, $p = 0.690$) in study groups. Generally hemoglobin showed significant positive correlation with height, weight and fasting blood glucose in the study group and positive correlation with age, weight, body mass index and fasting blood glucose in the control groups.

Fasting blood glucose also had a significant positive correlation with age ($r = 0.631$, $P = 0.021$), height ($r = 0.165$, $P = 0.207$) and weight ($r = 0.343$, $P = 0.007$) in the severe malaria patients as shown in Table 7. Additionally; it was observed that platelets value of the control group correlated negatively with age, height, weight, body mass index and fasting blood glucose and also it is not statically significant. There was correlation between hematological measurement and anthropometric measurements with p value > 0.05 for both control group and severe malaria patients of *Plasmodium falciparum*.

As indicated in Table 7 the socio demographic and anthropometric parameters factors were significantly associated with platelets except height. It could be predicted that the association between severe malaria patients and hematological parameters is influenced by demographic and anthropometric parameters factors. Thus, based on the statistical data, age is a factor that causes any significant differences between severe malarial.

Table 7:- Adjustments of Pearson correlation co-efficient among anthropometric, biochemical and hematological indices on severe malaria patients and control groups at Gambella General Hospital, Ethiopia, November, 2016 to February, 2017.

Hemoglobin (Hb) level correlation between study and control groups.

	Age	Height	Weight	BMI	FBG	Age control	Height control	Weight control	BMI control	FBG control
R	0.108	0.281*	0.144	-0.052	0.290*	0.609**	0.069	0.675**	0.560**	0.772**
P	0.410	0.030	0.273	0.690	0.025	0.000	0.672	0.000	0.000	0.000

Platelets (PLT) level correlation between study and control groups.

	Age	Height	Weight	BMI	FBG	Age control	Height control	Weight control	BMI control	FBG control
R	-0.052	-0.043	-0.107	-0.091	-0.033	0.266	-0.050	0.131	0.136	0.322*
P	0.691	0.745	0.415	0.487	0.804	0.097	0.760	0.422	0.404	0.043

Fasting blood glucose (FBG) correlation between study and control groups.

	Age	Height	Weight	BMI	Age control	Height control	Weight control	BMI control
R	0.298*	0.165	0.343**	0.247	0.631**	0.307	0.723**	0.447**
P	0.021	0.207	0.007	0.057	0.000	0.054	0.000	0.004

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Bi-variant analysis correlation was used for the determination of the association between MCV and age in both severe malaria infected patients and non-infected groups. Using the line scatter plots measurements and shows a positive correlation between MCV and age and no significant (R = 0.122, P = 0.353) as shown in figure 3. There was an increase in MCV as age increased in both malaria infected and the non-infected groups. And a statistically non-significant positive correlation was observed the association between MCHC and BMI; therefore, it indicates marked increase in MCHC as BMI increased in both malaria infected and the non-infected groups as shown in figure 4.

Figure 3:-Scatter plot showing association between MCV and age at Gambella General Hospital, Ethiopia, November, 2016 to January 2017.

R = 0.122

P = 0.353

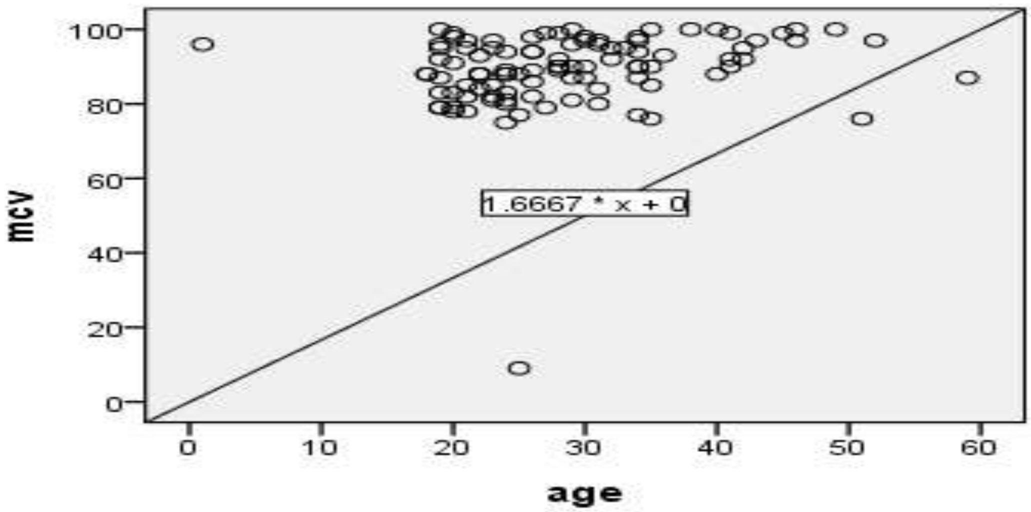
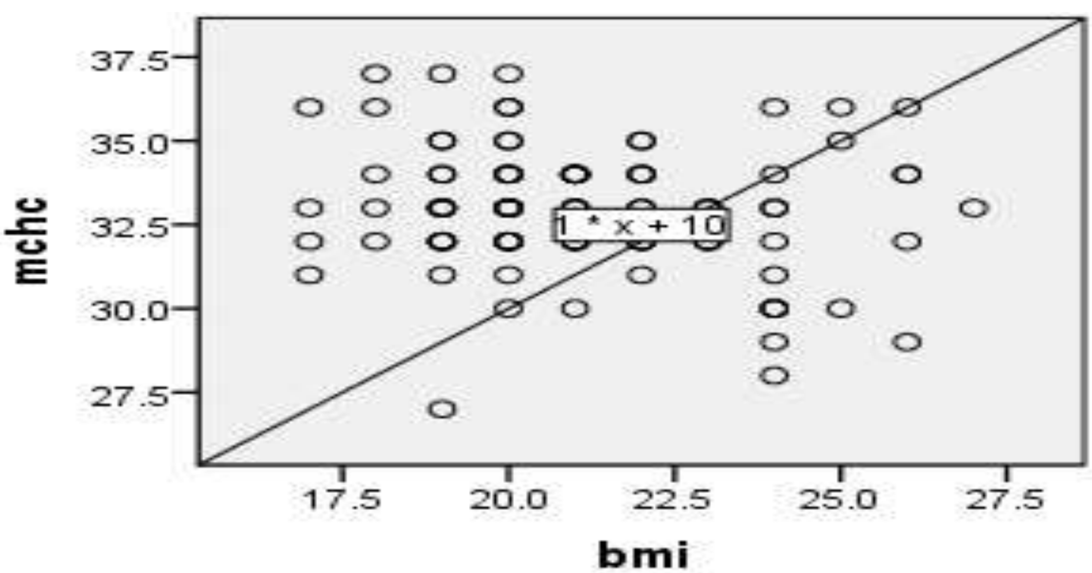


Figure 4:- Scatter plot showing association between MCHC and BMI at Gambella General Hospital, Ethiopia, November, 2016 to January 2017.

R = 0.22

P = 0.868



6. DISCUSSION

This study focused on evaluation and examination of blood biochemical parameters (fasting blood glucose) as well as hematological parameters (RBC, Hb, Hct, MCV, MCH, MCHC and PLT) in medically diagnosed severe *Plasmodium falciparum* malaria patients. Changes in hematological and biochemical parameters among severe malaria infected patients have been reported. Different study also reported many hematological and biochemical abnormalities caused by severe malarial infection of *plasmodium falciparum*. The main findings of the present study were that thrombocytopenia, anemia and decrease in the fasting blood glucose were the predominant manifestations of severe *Plasmodium falciparum* malaria patients. Severe malaria causes a major public health problem in people living in the highly affected areas of Gambella Region, South Western Ethiopia. Evidences presented in this work suggest that hematological and biochemical parameters were altered in severe *Plasmodium falciparum* malaria infected patients.

Some hematological parameters such as red blood cells, hematocrit, hemoglobin and platelets were decreased as the density of parasite of the patients rises of severe malaria. The present study also reports findings of the biochemical and hematological parameters as well as anthropometric indices with respect to severe malaria and their implication as risk factors of the diseases. According to this study, the major hematological changes include, notably lower values of RBC, Hb, Hct levels and platelet count and other platelet abnormalities among other parameters generated from complete blood count resulting in disseminated intravascular hemolysis. Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications (Khan et al, 2012). Thrombocytopenia was detected in these patients with complicated severe malaria caused by *Plasmodium falciparum*. This is a common hematological alteration in severe malaria, which may be a result of peripheral platelet destruction and consumption. Studies showed that immune complexes generated by malarial antigens lead to sequestration of the injured platelets by macrophages in the spleen (Bakhubaira, 2013).

In this study, red blood cells, hemoglobin (Hb) and packed cell volume (PCV) decreased in severe *Plasmodium falciparum* malaria patients compared to the healthy subjects. The mean hemoglobin concentration showed 11.3 g/dl in *Plasmodium falciparum* affected patients. One study done in Kenya reported that a significant reduction in red blood cells, hemoglobin concentration, hematocrit and palates in patients with severe malaria. Reduced red blood cells and hemoglobin in severe malaria may be attributed to the increased breakdown of red blood cells by the parasites. It has also observed that anemia is more common amongst the patients infected by *Plasmodium falciparum* (Al-Salahy *et al*., 2016). Furthermore, severe malarial anemia is defined as concentration of Hb < 7 g/dl. Therefore, the drop in hemoglobin concentrations in the malarias subjects in this study ranged between 8 and 15 g/dl, ranging approximately from mild to moderate anemia.

The present study reports a significant reduction in red blood cells, hemoglobin, hematocrit and platelets level of individuals infected with severe malaria of *Plasmodium falciparum* as compared to those control groups as shown in Table 5. This significant decrement might be due to changes in the RBC antigen structure caused by the parasitic invasion that stimulate the production of antibodies to RBCs, which triggers immune-mediated RBC lysis. Similar results were also presented in one study that demonstrated significant difference and decrease in Hct levels in febrile illness between control groups and severe malaria patients of male adults. indicating that reduction in most RBCs indices might be involved. This because of the malaria parasite caused excessive destruction and reduced production of red blood cells and hemoglobin (Abro *et al*., 2008, Sirak *et al*., 2016).

Another study conducted in Aden University of Yemen also agrees with this study where hemoglobin concentration showed lowest mean values reaching 3.5 g/dl in patients with severe anemia. *Plasmodium falciparum* malaria is one of the most common causes of anemia. Multiple mechanisms cause anemia in severe malaria, the most important being hemolysis of infected and uninfected red blood cells (RBCs), splenic sequestration of RBCs, dyserythropoiesis, and bone marrow suppression; such factors can culminate in the chronically low hemoglobin values observed in patients residing in endemic regions (Bakhubaira, 2013, Geleta and Ketema, 2016).

6.1. Levels of biochemical parameters (fasting blood glucose) in severe malaria patients.

This study show that severe malaria infection of *Plasmodium falciparum* caused significant reduction in fasting blood glucose (FBS) levels from (Table 4). Fasting blood glucose level was measured for controls as well as for the severe malaria patients and the results showed that there was a significant difference between the two groups. The severe malaria patients had lower average value of fasting blood glucose level (91.60± 13.431 mg/dl) than the control group (111.05 ± 10.208 mg/dl) with a p-value < 0.01. This shows that the fasting blood glucose level had really gone down for the severe malaria patients than control group may be due to severe malaria of *Plasmodium falciparum*.

The significant difference in fasting blood glucose between the severe malaria patients and the control groups is due to the different factors like; increased consumption of glucose by the host and the growing parasites, increased peripheral requirement of glucose consequent upon anaerobic glycolysis, reduced absorption capacity for sugar transport both actively and passively, increased metabolic demands of febrile illness, obligatory demand of parasites and failure of hepatic gluconeogenesis and glycogenolysis (parasites consume up to 70 times as much glucose as uninfected cells) (Patel *et al*, 2003, Thien *et al*, 2006).

In one study *Plasmodium falciparum* induced reduction in the blood glucose which could be due to the invasion of the liver cells by the malaria parasite that can cause organ congestion, sinusoidal blockage and inflammation of pancreatic cells. This in turn leads to increased intracellular insulin accumulation and slow receptor recycling. Still in another study conducted in Aden University Yemen *Plasmodium falciparum* from the total (77) of severe malaria patients 5(6.5%) were hypoglycemia, where as the rest were normal (Bakhubaira, 2013). Similar study was also conducted at M.G.M. Medical College & M.Y. Hospital, Indore (M.P.) shows fasting blood Sugar less than 40 mg was noted in 6.73% severe malaria patients, which is similar to the present study (Khuraiya *et al*., 2016).

6.2. Levels of hematological parameters in severe malaria patients.

This study demonstrated some hematological parameters in complicated severe malaria due to *Plasmodium falciparum*, which is the most common type of malaria infection in Gambella region, accounting for 90% of malaria cases. It included data on 60 confirmed severe malaria of *Plasmodium falciparum* subjects and 40 controls groups. The study mainly observed hematological parameters of severe malaria positive subjects compared to the control groups. There were decreased RBC, Hb, Hct and PLT in severe *Plasmodium falciparum* malaria confirmed subjects as shown in Table 4. Malaria parasitemia and level of hematological parameters were also evaluated in our study. This study reports that RBCs, Hct , Hb and platelet count were found to be significantly reduced in patients with high parasitemia of *Plasmodium falciparum* of ($P < 0.01$).

Malaria infection remains a serious health problem in sub-Saharan Africa including Ethiopia specifically in Gambella Region which is endemic. The present comparative cross sectional study was conducted at Gambella Region, South Western of Ethiopia between November, 2016 to February, 2017, in order to study the alteration in hematological parameters during severe malaria of *Plasmodium falciparum*. Hematological changes have been associated with severe malaria infection and these have been found to involve red blood cells, hemoglobin hematocrit and thrombocytes. The present study showed that the severe malarial infection affects hematological parameters in severe *Plasmodium falciparum* patients than Control groups. In this study, anemia and thrombocytopenia emerged as a predictor of severe malaria which was an observation of many studies which confirm it. Low red blood cells, hemoglobin, hematocrit and platelets were a finding of severe malarial patients and thrombocytopenia and anemia was common in the severe malaria infections.

6.2.1. Association between anemia and severe malaria

Anemia is a hematological disorder characterized by the reduction of the hemoglobin level to below the reference range depending on age, sex and altitude (Kosiyo, 2016). Severe malaria patients were found anemic as observed from the low red blood cells counts below $4.5x10^6$cells/µl. Therefore, according to Table 4, 45% had mild anemia, 13.33% moderate anemia, 41.66% were normal and there is no severe anemia in the severe malaria patients of *Plasmodium falciparum.* The mean value of RBCs count was significantly lower in patients with *Plasmodium falciparum* malaria ($4.42x10^6$/µl) than control groups ($5.73x10^6$/µl) (P value < 0.01) as shown in Table 4. In one study done in Medical College of Rajkot (Gujarat) is similarly decreased RBC count which was seen in 83.5% in a severe malaria of *Plasmodium falciparum*. Thus RBC count reduction was strongly associated with *Plasmodium falciparum* infection which was subsequently responsible for severe anemia in patients. Other study out of the 100 cases studied 91% had low hematocrit compared to 9% normal hematocrit (Agravat and Dhruva, 2010).

In this study, significantly lower values of red blood cells, hematocrit and hemoglobin concentration have been observed from the hematological parameters in severe *Plasmodium falciparum* malaria infected adult males compared to control groups. In various other studies hemoglobin was significantly reduced among malaria patients, which can be due to increased breakdown of red blood cells by the parasites. These lower values might be due to the variety of morphological changes exhibited by RBCs, malaria-associated changes in composition of plasma, mechanical destruction of infected RBCs, bone marrow suppression, accelerated RBC destruction, removal by the spleen in conjunctions with ineffective erythropoiesis, dyserythropoiesis, intravascular hemolysis caused by non-immune destruction of infected RBCs and decreases the production of erythrocytes in case of high parasitemia (Bakhubaira, 2013, Godse, 2013, Sirak *et al*., 2016, Reddy *et al*., 2016).

The lower values of red blood cells and hemoglobin caused by malaria parasites which can develop within, invade red blood cells (erythrocytes) and consume up to 75% - 80% of their hemoglobin as nutrient source and reduced production of RBCs. Potential causes of hemolysis include loss of infected cells by rupture or phagocytosis, removal of uninfected cells due to antibody sensitization or other physicochemical membrane changes and increased reticuloendothelial activity, particularly in organs such as the spleen (Okwa, 2012, Meraiyebu and Akintayo, 2012).

The results in this study showed that there was a significant difference between the hematocrit values of control adult males and positive severe *Plasmodium falciparum* malaria. This could be indicative that severe malaria parasites caused excessive destruction and reduction in production of red blood cells. A retrospective study was conducted in Phop Phra Hospital, a hospital located in the Thailand-Burma border area; a malaria-endemic area, between 2013 and 2015 partially similar with the present study results RBCs, Hb, Hct, MCV, MCH and MCHC were significantly lower in patients with severe malaria. This may be due to the parasite‟s primary target being RBCs, resulting in an accelerated removal of both parasitized and non parasitized RBCs by the destruction of RBCs and bone marrow dysfunction (Kotepui *et al*., 2017). One report also indicated that a high level of malaria parasitemia was associated with high RBC destruction. Anemia (Hb level < 11 g/dL) was frequently found in malaria positive patients, especially patients infected with the *Plasmodium falciparum*. The hemoglobin values of these two groups when compared had P values less than 0.01 which was statistically significant. This could be due to hemolytic mechanisms and accelerated removal of both parasitized and non-parasitized red blood cells and ineffective erythropoiesis (Abdalla, 2000, Khuraiya *et al*., 2016).

In a similar study the following parameters were significantly lowered in malaria-infected patients; red blood cells (RBCs), hemoglobin (Hb), platelets count while mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were higher in comparison to non-malaria infected patients ($P < 0.05$). This may be the reason why the rate of RBC production leads to the release of immature RBCs into blood circulation, which may cause an increase in the values of MCV, MCH, and MCHC (Kotepui *et al*., 2014). And one study done in Gambella region, at Gambella General Hospital deals no significant mean difference observed in values of MCV, MCH and MCHC of hematological parameters (Sahle *et al*., 2017).

6.2.2. Association between thrombocytopenia and severe malaria

Thrombocytopenia is a hematological disorder resulting in the reduction of the number of platelets in the peripheral blood to below the reference range. The normal platelet count is 150 –

$400x10^9$ cells/l (Kosiyo, 2016). Both qualitative and quantitative platelet abnormalities occur in severe malaria. In this study, platelet counts were markedly reduced in severe *Plasmodium falciparum* malaria infected patients compared to non-malaria infected control groups as shown from Table 4. Reduction in the number of platelets was one of the well-known hematologic changes observed in patients with severe malaria. One study reports platelet count changes are particularly associated with severe *Plasmodium falciparum* malaria. Increased the number of megakaryocytic in the bone marrow leads to decreased thrombopoiesis an unlikely cause of thrombocytopenia in malaria (Martínez-Salazar, 2014).

Severe malaria infected patients also have high levels of specific IgG in their blood which binds to platelet-bound malaria antigens possibly leading to accelerated destruction (Moulin *et al.*, 2003). Due to bleeding in *Plasmodium falciparum* malaria the platelet count is typically reduced. Nevertheless, bleeding due to thrombocytopenia is very rare in malaria and also spontaneous bleeding is rare in the adults. When it develops, it results from disseminated intravascular coagulation (DIC). Furthermore, consumption of platelets by the process of DIC was also another factor that contributed to thrombocytopenia in complicated *Plasmodium falciparum* malaria. Platelet hyperactivity followed by platelet hypo activity was also the two major changes in platelet function during malarial infection that causes decreased platelet count. Generally bleeding and coagulation abnormalities are seen only in limited number of patient with severe *Plasmodium falciparum* malaria. This can be due to thrombocytopenia and/or disseminated intravascular coagulation (DIC) (Kidist, 2015).

In another study conducted at Arba Minch health center located in the Gamo Gofa Zone of the Southern Nations Nationalities and Peoples Region in January 2016, the prevalence of thrombocytopenia is significantly associated with malaria infection though the mechanism of reduction in platelet count among such patients was not fully understood. Immune complexes generated by malaria antigen might lead to sequestration of the injured platelets by macrophages in the spleen and platelet consumption in disseminated intravascular coagulation together with platelet dysfunction resulting in hyper aggregation are thought to contribute to thrombocytopenia in malaria. Apparently, platelet activation by *Plasmodium falciparum* can potentially lead to the formation of micro-aggregates of infected red blood cells and platelets which can occlude blood vessels and it also leads to binding to and activation of the endothelium (Reddy *et al*., 2016, Mikre and Zerdo, 2016).

The results in this study showed mild thrombocytopenia was observed in 63.3% (n = 38), 5% (n = 3) moderate thrombocytopenia, 31.7% (n = 19) were normal and no severe thrombocytopenia seen in severe malaria infected patients as shown in Table 6. One similar prospective study was done at Aden Governorate with complicated severe malaria admitted to Al-Gamhouria Modern General Hospital in Aden, Yemen (2010-2011) that included 77 patients, Thirty-three patients from the studied 77 with severe complicated malaria had thrombocytopenia (platelet count of < $150x10^9$cells/l) at variable levels. Mild thrombocytopenia with counts of 75 to $150x10^9$cells/l, moderate thrombocytopenia with counts of 50 to $75x10^9$cells/l and severe thrombocytopenia with counts of <$50x10^9$/l. The studied cases of thrombocytopenia were classified as 57.6% in mild, 24.2% in moderate and 18.2% in severe thrombocytopenia. Thrombocytopenia was detected in 42.9% of the studied patients with complicated severe malaria; however, none of them developed bleeding. This is a common hematological alteration in malaria, which may be a result of peripheral platelet destruction and consumption (Bakhubaira, 2013).

Malaria is commonly associated with various degrees of hematological changes like thrombocytopenia. Thrombocytopenia is a very common association with severe malaria. The two major changes of platelet counts during severe malarial infection are in thrombocytopenia and platelet dysfunction. Thrombocytopenia has been observed in 60–80% of both *Plasmodium vivax* and *Plasmodium falciparum* infection. The mechanism of thrombocytopenia in severe malaria is due to peripheral destruction and consumption (Kidist, 2015). The incidence of thrombocytopenia was more common among patients suffering from complicated *Plasmodium falciparum* malaria. The highest thrombocytopenia occurs at 5-6 days after infection and become normal five to seven days after parasite clearance. During malarial infection, despite the presence of thrombocytopenia, the number of megakaryocytes in the bone marrow remains adequate or increased (Khan *et al*., 2012, Mikre and Zerdo, 2016).

Additionally, the common hematological alteration in severe malaria patients may be a result of peripheral platelet destruction and consumption. Other studies also showed the immune complexes generated by malarial antigens lead to sequestration of the injured platelets by macrophages in the spleen. Suggested that the mechanisms of thrombocytopenia in malaria include splenic phagocytosis of activated platelets, coagulation disturbances and bone marrow alterations (Bakhubaira, 2013, Birhanu *et al*., 2017). The risk of thrombocytopenia was more in *Plasmodium falciparum* than *Plasmodium vivax.* Prevalence of severe thrombocytopenia in *Plasmodium falciparum* infection was higher than *Plasmodium vivax* infection. But, the moderate thrombocytopenia was seen to be higher in *Plasmodium vivax* than *Plasmodium falciparum* infection (Kidist, 2015).

In another studies also existed statistically significant differences in the number of platelets among malaria-infected patients compared to control groups. Evidences support that thrombocytopenia was observed in a majority of patients with severe malaria patients and also observed that at high parasitemia, platelets were found to be significantly lower. Similar studies also show increasing levels of *Plasmodium falciparum* parasite density result in a decreased platelet count. This significant reduction might be due to hyper-reactive splenomegaly, coagulation disturbances, bone marrow alterations, immunologically mediated platelet destruction, oxidative stress, the role of platelets as cofactors in triggering severe malaria and increased consumption of the platelets in the periphery (Khan *et al*., 2012, Kidist, 2015, Sirak *et al*., 2016). Another similar study also conducted in King Saud University showed reduction in platelets between malaria patient group and the control group, and it was statistically highly significant ($p < 0.001$) (Al-Omar *et al.*, 2010).

In the present study hemoglobin positively correlated with demographic indicators, example; hematological parameters to ($r = 0.108$, $p = 0.410$) age of the study group of severe malaria patients. In studies conducted in Bangladesh, Myanmar, India, and Indonesia from June 2003 through May 2005 on anemia. Severe anemia (hemoglobin < 7g/dl) was present in 180 (18.4%) of 977 patients; the prevalence of anemia decreased stepwise with age ($P < 0.01$) (Dondorp *et al*., 2008).

Hemoglobin positively correlated with demographic indicators of the hematological parameters to ($r = 0.108$, $p = 0.410$) age in study groups. However, there is negative correlation with body mass index of (r = -0.052, p = 0.690) study groups. Hemoglobin also showed a positive correlation with height, weight and fasting blood glucose in the study group and positive correlation with age, height, weight, body mass index and fasting blood glucose and statically significant with all anthropometric and demographic indicators in the control groups. A study conducted in Jharkhand in the eastern region of India, a significant correlation in the hemoglobin and blood sugar level was observed in *Plasmodium falciparum* malaria infection with an increase of age and they were found significant as r = 0.136, P = 0.01 and r = 0.196, p = 0.003 respectively. Interestingly, hemoglobin is positively associated with age (Pearson r = 0.369, p = 0.004), the blood sugar also positively associated with age (Pearson r = 0.308) in the case of *Plasmodium falciparum* infection (Hussain *et al*., 2013).

Generally hypothesized on the basis of our interesting and influenced observation during this study the fasting blood sugar was significantly correlated with age in the case of *Plasmodium falciparum* infection of severe malaria. In one study age is significantly correlated with hemoglobin in the case of *Plasmodium falciparum* infection, therefore, these hematological and biochemical parameters may be used as a marker of disease severity and of diagnostic potential during malarial infection (Hussain *et al*., 2013).

There was correlation between platelets measurement level with demographic and anthropometric measurements like age, height, weight, body mass index with p value > 0.05. This result is supported by study conducted in the Medical Technology Laboratory Unit, between January first, 2009 and January first 2012 at Phobphra Hospital Tak Province, it could be predicted that the association between malarial parasitemia and hematological parameters is not influenced by age. Thus, based on the statistical date, age is not a factor that causes any significant differences between malarial parasitemia and hematological variables. Age correlated with risk of malaria infection and showed that the association between thrombocytopenia and malaria infection would not be significant following adjustment for age of patients. However, adjustment in these demographic variables did not confound the association between thrombocytopenia and malaria (Kotepui *et al*. 2014).

7. CONCLUSIONS

Malaria is one of the most serious vector-borne diseases transmitted by the bite of infected *Anopheles* mosquitoes which carry the *Plasmodium* parasites. Severe malaria infection is an important factor that alters hematological and biochemical parameters indices in patients. Hence, those altered biochemical and hematological parameters are associated with acute severe *Plasmodium falciparum* malaria complications and are identifiable in addition to malaria parasite detection. *Plasmodium falciparum c*an cause significant hematological changes with high incidence of thrombocytopenia and anemia. Adult males infected with severe *Plasmodium falciparum* malaria exhibited important changes in some biochemical and hematological parameters with low fasting blood glucose, RBC, PLT count, Hcr and Hb concentration. Mean values of hematological profiles were significantly different in the two groups. They were the most important predictors of severe malaria infection in the study area. Anemia and thrombocytopenia are the major hallmarks that are associated with complication of severe malaria. The thrombocytopenia could lead to many undesired complications of severe malaria and hence it also requires due attention. Decrease in RBC, Hb, Hct and PLT were important predictors of severe malaria where as MCV, MCH, MCHC were not significantly decreased and can be used in diagnosis along with clinical manifestation. Hypoglycemia is also a very common phenomenon among severe *Plasmodium falciparum* malaria patients that requires attention during patient treatment. This will enhance the determination capability of severity of malaria disease at diagnosis and reduces delay in medical intervention required to avoid mortality arising from malaria complication. Further studies with larger sample size from other settings are needed to substantiate the findings.

8. STRENGTHS AND LIMITATIONS OF THE STUDY

8.1. Strengths of the study

The strengths of this study are that it includes several demographic, clinical and anthropometric parameters claimed to be associated with the variables under study. In addition, the anthropometric indicators are measured directly than by self-report. Results from this study were compared to current evidence based practice guidelines for the management of malaria patients at complicated risk screening studies performed nationally and internationally. Well-informed alterations in blood parameters in severe *Plasmodium falciparum* malaria infection enable the clinician to establish reliable diagnosis and therapeutic interventions.

8.2. Limitations of the study

The factors that may affect hematological parameters, such as micronutrient deficiencies, genetic and ethnic background of patients are not taken into account during the study. The study was only a cross-sectional study, and no generalization can be attempted for the Ethiopian population as a whole. Moreover, factors such as malnutrition, iron deficiency, bone marrow dysfunction are also not taken into account during this study. Hematological changes as a consequence of bacterial, viral and helminthes infection in the patients studied were not taken into account during clinical screening. Underestimation of malaria parasitemia could have occurred as the study used only microscopical methods of detection and no attempt was made to detect through molecular biological techniques, which was not available at Gambella region. Meaning that, some malaria negative patients could have had malarial parasites, which could have been detected by PCR only and this could have lead to an underestimation of the effect of malarial parasitemia of *Plasmodium falciparium* on hematological parameters. History of the exposure due to travel in the region was also not recorded and lack of any previous studies in the region restricted any reliable comparisons arising from variable transmissions. The study also could not compare the effects of hematological profiles variations in dietary habits.

9. RECOMMENDATIONS

Investigations on biochemical and hematological parameters were very important in estimating the density of malaria parasite and performing hematography to assess the variety of hematological changes exhibited by RBC, Hb, Hct and PLT associated with severe *Plasmodium falciparum* malaria infection.

The prevention of malarial infection and early diagnosis were the measures likely to decrease acute severe malarial hematological parameter changes in developing countries. Early prompting diagnosis and measurement of the different biochemical and hematological parameters can reduce mortality and enhance recovery from the severe malaria disease. These findings offer the opportunity to recognize and diagnose malaria related hemolytic anemia and help support the treatment of this, as well as relieve symptoms of severe malaria in endemic regions of Gambella. The frequency of anemia and thrombocytopenia in acute severe malaria infections is common enough to justify the inclusion of complete blood count early in the management protocol of this common and potentially fatal disease in order to reduce the mortality and morbidity associated with it. It is further recommend that the treatment guidelines regarding thrombocytopenia and anemia be also included in the management. Furthermore creation of public awareness as well as clinical care should be intensified among severe malaria patients in the Gambella Regions of Ethiopia, regarding the risk factors for severe malaria so as to control morbidity and mortality in the malaria patients.

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11. APPENDICES

Appendices I: Procedures for Analysis

Blood collection for automated count

Capillary blood in Pre-cleaned slides were labelled (preferably at the frosted-end) with the patient‟s name (or other identifier) and date and time of collection. The site was cleaned well with 70% alcohol and allowed to air dry. A deep prick was then be made at the side of the side of the pulp of the 3rd or 4th finger and the first drop of blood wiped away with clean gauze and squeezed to obtain a free flow of capillary blood into 1.5 ml EDTA vacutainer tube and a clean dry swab to be applied to prevent excessive bleeding as the content of vacationer tube is properly mixed to prevent clotting.

Venous blood in adults

Median cubital vein was cleaned with 70% alcohol swab and allowed to air dry. A 21 guage needle attached to a 4 mls syringe was inserted into the median cubital vein inclined at an angle of 25° with the bevel facing up and plunger pulled backwards to obtain 4 mls of blood and pressure applied using a dry swab to prevent excessive bleeding. The blood was then transferred to a 5mls EDTA vacutainer tube and mixed thoroughly to prevent clotting.

Automated Blood count

Automated blood count was preceded by mixing of anti-coagulated whole blood in a mixer. The patient details e.g. name, lab number, age and sex were fed manually through the touch screen monitor of coulter counter followed by putting the vacutainer tube into the tube holder. This was followed by pressing the run button as the automated counter does the self-priming, sizing, enumeration blood cell and calculation of blood cell indices. The results were automatically displayed on the coulter counter screen and then recorded from the machines.

Procedure for thick and thin smear

Both thick and thin smears were made on the same slide. The frosted end of the slide was labeled with the patient number and a drop of blood put next to the frosted end and another smaller drop at the middle of the slide. The first drop was spread using a corner of the spreader in one circular direction to make even thick film, of a 1 cm diameter in size. For the thin film the spreader slide was placed in contact with second drop at an angle and gently pushed backwards to obtain even spread of blood and then pushed gently towards the other end of the slide. The slide was then allowed to air dry.

Giemsa staining procedure

The smears were put on a flat staining rack to air dry or light-dry. Giemsa stain of 10% dilution was used to flood the air dried slides alongside the quality control slides for 10-15 minutes. This step was followed by washing the slides using phosphate buffer of pH 7.2 then allowed to air dry and examined using oil immersion objective.

WHO Grading of MPs

The WHO grades malaria parasitaemia upon examination of slide through battlement method as follows

I. 1-10 parasites per 100 high power fields......................+ or (PF^{+1})

II. 11-100 parasites per 100 high power fields..................++ or (PF^{+2})
III. 1-10 parasites per high power field.............................+++ or (PF^{+3})
IV. >10 parasites per high power field..............................++++ or (PF^{+4})

Appendices II: Information sheet

Research Project: - Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region, in South Western Ethiopia.

Sponsoring organization: **-** Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: - Hadera G/krstos Kidu (MSc in biochemistry candidate)

Advisors: **-** Solomon Genet (PhD), Menakath Menon (PhD)

Objective of the research project: - To compare the change in biochemical parameter and hematological parameters among male patients infected with severe *Plasmodium falciparum* and controls groups in Gambella region in an endemic region of malaria in South Western Ethiopia.

1. Purpose of the study

Malaria is caused by parasites that are transmitted by mosquito bites. Mosquitoes can become infected with severe malaria after they bite humans who are carrying malaria parasites specially *Plasmodium falciparum* and this leads to change in fasting blood glucose levels and hematological parameters level. Studies the changes in fasting blood glucose levels and level in hematological parameters patients suffering of severe malaria, to investigate the effects of severe malaria caused by infections of *Plasmodium falciparum* of severe malaria patients and to identify the role of fasting blood glucose and hemoglobin in severe malaria pathogenesis which caused by the *Plasmodium falciparum.*

2. Study procedures

You or your patients will be asked to donate a small finger prick blood sample at the beginning of the study. I use the sample to determine the presence of *Plasmodium falciparum* malaria parasites in the blood and to determine on the changes in fasting blood glucose levels and blood level in hematological parameters patients suffering of severe malaria, to investigate the effects of severe malaria.

All individuals who have a fever and have a positive test for malaria *Plasmodium falciparum* (Microscope) will be treated.

3. Voluntary participation

Your decision not to participate or to withdraw from participation will not affect the care you or your patient will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time. If you chose not to participate, you have access to the same level of clinical care.

4. Discomforts and Risks

You or your patients might feel a small amount of discomfort during blood sampling taking and you or your patients may have a small amount of bruising or bleeding where the blood sample was taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is four ml and too small to influence you or your patients health and the blood will quickly be replaced by your/your patient‟s s body.

5. Benefits

You or your patients will receive free clinical care for the duration of the study. Medical examination and temperature measurements will be repeated on a daily basis by trained field workers until blood taken and you or your patients knows your fasting blood glucose and blood hematological parameters.

6. Confidentiality statement

The records concerning your/your patients" participation are to be used only for the purpose of this research project. Your/your patients" name will be used on labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, I will give you/your patients a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential and under lock and key. Only senior members of the study team will have access to information linking your/your child"s name with your/your patients study number.

7. Questions and freedom to withdraw from the study Freedom to ask questions

If you have questions regarding this study or would like to be informed of the results after its completion, please do not hesitate to contact: Hadera G/krstos, School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia.

Cell phone:-0912686166

Email:hadera.gk11@gmail.com

Appendices III: Informed consent agreement for adult males (18-55 years)

Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region in South Western Ethiopia.

I, ____ (participants name and sign), having full capacity to, do here by consent to my participation in the research study entitled Studies on biochemical parameters (fasting blood glucose) and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region , in South Western Ethiopia by "Mr. Hadera G/krstos". The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it was conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by ___________________________, and are set forth in the Informed Consent Explanation, which I have signed. I have been given an opportunity to ask questions concerning this investigational study, and any such questions have been answered to my full and complete satisfaction. If there are any further questions that may arise, I may contact by Mr. Hadera G/krstos (0912686166) School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia. I understand that I may at any time during the course of this study revoke my consent and withdraw my participation from the study without prejudice; however, I may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary my wellbeing.

I understand / do not understand the practical consequences of this study, asking me to provide weekly small blood samples and measuring fasting blood glucose and the different hematological parameters.

Appendices IV: Questionnaire (English version)

Title of the study: Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region, in South Western Ethiopia.

This questionnaire-based interview will be fed into and data will be collected using questionary paper. The interviewer begins by introducing himself and the purpose of the study to the participants, requesting the respondent‟s for participation in the survey, thanking the participant for giving consent to ta/ke part in the study.

Date: __ __/__ __/__/ __ __ __ (dd/mm/yyyy)

1. Patients name: ____
2. Participant‟s Card number: _____________________________
3. Participant‟s telephone number: ________________________
4. Date of Birth: __ __/__ __/__ __ __ __(dd/mm/yyyy)
5. Age (in years)_____________________________years
6. Wereda (District) Name:______________________________________
7. Name of town or village: ___________________________________
8. What is the nearest health facility to your house?
   a. Hospital   c. Clinic
   b. Health center   d. Health post
9. Type of parasite: _________________________________
10. Height in meter:_________________________________
11. Weight in kg:____________________________________
12. Body mass index(BMI): _____________________
13. Blood glucose-------------------------mg/dl or ------mmol/l
14. Levels of hematological parameters
    a. RBC= __________________________________
    b. Hb = __________________________________
    c. Hct=__________________________________

d. MCV=________________________________

e. MCH=________________________________

f. MCHC=_______________________________

g. PLT=_________________________________

15. Have you had malaria in the last three weeks?

a. Yes b. No c. Do not know

16. If Yes, when exactly? ____

17. Was treatment given before?

a. If yes, name of the drug if known___________________________________

b. No c. don‟t know

18. Do you have a history of Anemia?

a. Yes b. No

19. Is there had any hypoglycemia or hyperglycemia before?

a. Yes b. No c. Normal blood sugar

20. Are you taking anti-malarial drug currently?

a. Yes b. No

21. Time of infection

a. 1-2 days b. 2-3 days c. 3-5 days d. 4- 6 days

Appendices V: Informed consent agreement for adult males (18-55 years) (Amharic version)

Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region, in South Western Ethiopia.

እኔ,____(ተሳታፊዎች ስም እና ፊርማ)
Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region, in South Western Ethiopia, በሚል ርዕስ በጥናቱ ውስጥ ከተሳትፎዎች ጋር ስምምነት በማድረግ በሙሉ አቅም "በደቡብ ምዕራባዊ ኢትዮጵያ ውስጥ በጋምቤላ ክልል አካባቢ, በአቶ ሀደራ ገ/ከርስቶስ ,የእኔ በፈቃደኝነት ተሳትፎ, ባህሪ, ቆይታ እና ዓላማ ያለው አንድምታ; ይህ ጥናት ነበር ይህም በዘዴዎች እና መንገዶች; እኔ ገብቶናል ይህም ምክንያታዊ ________________ ለእኔ እንዲህ ሊሆን የሚጠበቅ ሊሆን ይችላል, እና በበቂ መረጃ ስምምነት ማብራሪያ ውስጥ በተቀመጠው መንገድ ናቸው። ያለውን ችግሮች እና አደጋዎች, እኔ ይህን ጥናት በተመለከተ ጥያቄዎችን ለመጠየቅ አጋጣሚ ተሰጥቷቸዋል, እና ማንኛውም ያሉ ጥያቄዎች የእኔን ሙሉ የተሟላ እርካታ ድረስ መልስ ያሎዉ ተሰጣቸውም። ሊነሱ የሚችሉ ማናቸውም ተጨማሪ ጥያቄዎች ካሉ, እኔ አቶ ሀደራ ገ/ከርስቶስ በማነጋገር እና መዌያየት ይችላሉ(0912686166)። የህክምና ትምህርት ቤት, ባዮኬሚስትሪ ትምህርት ክፍል, በአዲስ አበባ ዩኒቨርሲቲ, የኢትዮጵያ መምሪያ እኔ በዚህ ጥናት ውስጥ ያለውን አካሄድ ወቅት በማንኛውም ጊዜ ያለኝን መሻር እና ጭፍን ያለ ጥናት ከ ያለኝን ተሳትፎ ማውጣት እንደሚችል ተረድቻለሁ; ወደ ሐኪም አስተያየት ውስጥ, እንዲህ ያሉ ፈተናዎችን የእኔ ደህንነት አስፈላጊ ናቸው, ከሆነ ግን, እኔ ተጨማሪ ምርመራ ሲያስገድዱ ሊጠየቁ ይችላሉ።

እኔ /ሳምንታዊ ትንሽ የደም ናሙናዎችን ማቅረብ እኔን በመጠየቅ እና ፆም የደም ግሉኮስ እና የተለያዩ Hematological መለኪያዎች ጋር ሲያመዛዝኑ በዚህ ጥናት ውስጥ ተግባራዊ ውጤት መረዳት ነዉ።

Appendices VI: Questionnaire (Amharic version)

<u>የጥናቱ ርዕስ</u>: Studies on biochemical parameters and hematological parameters among male population in a severe malaria (*Plasmodium falciparum)* infested area of Gambella region, in South Western Ethiopia.

ይህ መጠይቅ ላይ የተመሠረተ ቃለ ወደ መመዝገብ እና በጥያቅ ወረቀት በመጠቀም የሚሰበሰብ ይሆናል. በቃለ ስምምነት በመስጠት ለማግኘት ተሳታፊ እያመሰገነን በጥናቱ ላይ ተሳትፎ ለማግኘት የመልስ ሰጪ, በጥናቱ ውስጥ ክፍል መውሰድ እየጠየቀ, ራሱን እና ተሳታፊዎች የጥናቱ ዓላማ በማስተዋወቅ ይጀምራል።

ቀን: __ __ / __ __ / __ __ __ __ (ቀን/ወር/ዓ.ም)

1. ታካሚዎች ስም:______________________________
2. ተሳታፊ ካርድ ቁጥር:______________________________
3. ስልክ ቁጥር:________________________
4. የትውልድ __/__ /___(ቀን/ወር/ዓ.ም)
5. የዕድሜ (በዓመት)፡ ________________________
6. የወረዳ (ዲስትሪክት) ስም፡ __________________
7. ከተማ ወይም መንደር ስም: _____________________________________
8. ቤትህ ወደ ቅርብ ጤና ተቋሙ ምንድን ነው?

    ሀ. ሆስፒታል　　　　ሐ. ክሊኒክ

    ለ. ጤና ማዕከል　　　　መ. የጤና ልጥፍ ጥገኛ

9. ጥገኛ የታሃዋስያን አይነት:___________________________
10. ቁመት (በሜትር):__________________________________
11. ክብደት (በኪ.ግ):___________________________________
12. የሰዉነት ክብደት ልኬት (ኪ.ግ./ሜ^2):________________

13. የደም ግሉኮስ ------------------- mg/dl ወይም ------ mmol/l

14. **Hematological** መለኪያዎች ደረጃዎች

ሀ. RBC = ______________________________

ለ. HB = ______________________________

ሐ. Hct = ______________________________

መ. MCV = ______________________________

ሠ. MCH = ______________________________

ረ. MCHC = ______________________________

ሰ. PLT = ______________________________

15. ባለፉት ሶስት ሳምንታት ውስጥ ወባ ይዞህ ነበር?

ሀ. አዎ. ለ. አይ ሐ. አላውቅም

16. መልስህ አዎ ከሆነ, መቼ በትክክል?____________________________________

17. ህክምና በፊት ተሰጠው? ዕፅ ስም ሚታወቅ ነዉ?_______________________

ሀ. አዎ ለ. አላውቅም

18. ከዚህ በፊት የደም ማነስ ነበሮዎት?

ሀ. አዎ ለ. አልነበረኝም

19. ከዚህ በፊት የደም ስኳር ብዛት ዌይም ማነስ ነበሮዎት?

ሀ. አዎ. ለ. አይ ሐ. መደበኛ የደም ስኳር ነበረኝ

20. አሁን ጸረ-ወባ መድሃኒት በመውሰድ ነህ?

ሀ. አዎ ለ. አልወሰድኩም

21. ወባ ክያዝህ ስንት ቀን ሆነህ?

ሀ. 1-2 ቀናት ለ. 2-3 ቀናት ሐ. 3-5 ቀናት

The laboratory technicians performing a cytomorphological study on a peripheral blood film using a light microscope at Gambella General Hospital.