Sudan University Of Science And Technology

Collage Of Graduate Studies

Estimation of Malaria Parasite Density Using Actual and Assumed White Blood Cells Count in Khartoum State-Sudan

تقدير كثافة طفيل الملاريا باستخدام العدد الفعلي والعدد القياسي لكريات الدم البيضاء في ولاية الخرطوم-السودان

A dissertation submitted in partial fulfillment for degree of M.Sc. In Medical Laboratory Science (Parasitology and Medical Entomology)

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الآ ية

قال تعالى:

بسم الله الرحمن الرحيم

(وَمَا مُحَمَّدٌ إِلَّا رَسُولٌ قَدْ خَلَتْ مِن قَبْلِهِ الرُّسُلُ ۚ أَفَإِن مَّاتَ أَوْ قُتِلَ انقَلَبْتُمْ عَلَىٰ أَعْقَابِكُمْ ۚ وَمَن يَنقَلِبْ عَلَىٰ عَقِبَيْهِ فَلَن يَضُرَّ اللَّهَ شَيْئًا ۗ وَسَيَجْزِي اللَّهُ الشَّاكِرِينَ)

صدق الله العظيم

(سورة ال عمران -الاية 144)

Dedication

I dedicate this work

To my family

Who were very patient with me

Throughout this study.

To my teachers,

To my friends,

To my colleagues,

To my students,

To everyone who supported me

Acknowledgement

Firstly I am deeply thankfully to god for this favors. I gratefully acknowledge the help of my supervisor Dr. Ahmed Bakheet Abd Alla . My thanks extend to the staff of the department of parasitology and medical entomology (Sudan University Of Science and Technology) specially Dr Ali Alamin Nasir and those who helped me in hospitals and laboratory sites. They made things easier for me to work.

Abstract

This cross sectional study was conducted in Khartoum state during period from March to May 2017. The aim of this study was to determine malaria parasite density using actual white blood cell (WBC) and assumed WBC counts (8000/µl). Two hundred and fifty *Plasmodium falciparum* malaria-infected subjects was included in this study with age ranged between 1-67 years old with mean age (19)years old. Out of this 115 (46%) being males and 135 (54%) females. Blood samples were collected from all subjects. All samples were examined to count the asexual stages of parasite stained thick film. Total white blood cells were obtained by analyzing EDTA blood samples; using automated hematological analyzer system (Sysmex-KX2IN). Mean value of which was 5570/µl ±1659 . The parasite density were estimated by actual and assumed TWBCs to compare between the two methods. The study revealed disparity in parasite density that calculated by actual and assumed WBCs the mean of which (11346.7) and (16092.6) respectively in study population. This study were recommended to use actual white blood cells count instead of uses assumed WBCs count for estimate of the parasite density.

المستخلص

أجريت هذه الدراسة المستعرضة في ولاية الخرطوم خلال الفترة من مارس إلي مايو 2017. وكان الهدف من هذه الدراسة هو تحديد كثافة طفيل الملاريا *P. falciparum* باستخدام العدد الفعلي لكريات الدم البيضاء و العدد القياسي كريات الدم البيضاء (8000/µl). شملت هذه الدراسة عدد 250 مريض مصاب الملاريا *P. falciparum* تراوحت أعمارهم بين 1-67 رتع سنة وكان متوسط العمر(19) سنة 115 (46%) ذكور و135 (54%) الإناث. أخذت عينات الدم من الاشخاص الخاضعين لهذه الدراسة . تم عد الاطوار غير الجنسية لطفيل الملاريا عن طريق المسحة المصبوغة الثقيلة , وكذلك تم تعداد العدد الكلي لكريات الدم البيضاء بواسطة جهاز قياس الدم الآلي.(Sysmex-KX21N) حيث كان متوسط العدد الكلي لكريات الدم البيضاء 5570/µl ±1659. تم حساب كثافة الطفيل باستخدام العدد الفعلي والقياسي للمقارنة بين الطريقتين ,هذه الدراسة وجدت أن هناك تباين في كثافة الطفيل المحسوبة بالعدد الفعلي وبالعدد القياسي وكان متوسطها (11346.7) و(16092.6) على التوالي, في مجتمع الدراسة. توصي هذه الدراسة باستخدام التعداد الفعلي لكريات الدم البيضاء لحساب كثافة طفيل الملاريا بدلا عن العدد القياسي لكريات الدم البيضاء .

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Abbreviations

ACTs	Artemisnin-Based Combination
CDC	Central For Disease Control
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
ELISA	Enzyme Lined Immune Assays
HRP-Ⅱ	Histadine Rich Protein
HMS	Hyper Reactive Malaria Syndrome
IFA	Immune Fluorescent Assay
IgG	Immunoglobuline G
IgM	Immunoglobuline M
ITN	Insecticide Treated Mosquito Nets
IRS	Indoor Residual Spraying
MT	Malignant Tertian
PCR	Polymerase Chain Reaction
P.FALCIPARUM	Plasmodium Falciparum
PLDH	Parasite Lactate Dehydrogenates
QBC	Quantitative Buffy Coat
RDTs	Rapid Diagnostic Tests
SNP	Single Nucleotide Polymorphism
TWBCs	Total White Blood Cells
UV	Ultra Violet
WHO	World Health Organization

1-Introduction

1.1 Introduction

Malaria is the most important parasitic infection of man, it is associated with a huge burden of morbidity and mortality in many parts of the tropical world. Mortality rates increased among children referred to hospitals with severe malaria, although these rates are even higher in rural and remote areas where diagnosis and treatment are not readily available. The accurate diagnosis of malaria infection is important in order to reduce severe complications and mortality (CDC, 2014).

Malaria is a common and life-threatening disease in many tropical and subtropical areas . It is the commonest cause of hospital attendance in all age groups in Sudan, *P. falciparum* is the most predominant parasite specie accounting for about 98% of malaria cases in the Sudan (WHO, 20016).

Malaria is characterized by a stable, perennial, transmission in all parts of the country. The diagnosis of malaria and detection of severity of infection play the important role in control of disease. The parasite density provides information on the severity of infection and on the response to treatment(WMR 2015).

Diagnosis of malaria microscopically is routinely relied upon as a primary endpoint measurement of the level of malaria infection, this is expressed as parasite density and is classically defined as the number of asexual forms of parasite relative to a blood volume , White Blood Cells (WBCs) are relatively used in estimating *Plasmodium* parasitemia by

counting the number of parasites against a predetermined number of WBCs on Giemsa stained blood smears. Complete blood counts, particularly WBCs count, can be performed with new generation automated hematology analyzers and/or manually. Due to the frequent lack of facilities in some malaria endemic countries to quantify WBCs, an assumed WBCs count of 8000/µL of blood has been accepted by World Health Organization(WHO) as reasonably accurate to estimate malaria parasite densities. Assumed WBCs count of blood may generate systematic errors which could produce incorrect conclusions in patient management. The aim of this study to estimate the parasitemia using the assumed and actual TWBCs. Similar study done in central Ghana by Adu-Gyasi*et al.*(2012); this study use 10000\µl as assumed TWBCs. and found that not significant. Also in Sudan another study done in Eastern Sudan in children to determined the parasite density used assumed TWBCs 8000\µl and actual their result wasobtained from malaria parasitemia their found based on assumed is higher thanthe parasitemia based on actual TWBCs(Bilal*et al.*, 2015).

1.2 Rational

The WHO estimates that in 2015 malaria caused 212 million clinical episodes, and 429,000 deaths (WHO, 2016).

Each year approximately300–500 million malaria infections lead to over one million deaths, of which over 75 % occur in African children aged under five years infected with *P. falciparum*.

Quantification of parasite density is an important component in the diagnosis of malaria infection. The accuracy of estimation varies according to the method used.

Due to the frequent lack of facilities in some malaria endemic countries to quantify WBCs, an assumed WBCs count of 8000/µL of blood has been accepted by WHO as reasonably accurate to estimate malaria parasite densities. Assumed WBCs count may generate systematic errors which could produce incorrect conclusions in patient management.

The aim of this study was to assess the agreement between the parasite density values obtained with the assumed value of 8,000 cells/L and the accurate WBC count obtained from study population.

1.3 Objective

1.3.1 General objective:

To estimate malaria parasitedensity using actual and assumed white blood cell count in Khartoum state, Sudan.

1.3.2 Specific objective:

1. To estimate WBCs count in study population.
2. To compare the malaria parasite density among different age groups .
3. To compare malaria parasite density calculated by actual and assumed among study population.

CHAPTER TWO

Literature review

2.1 Background

Malaria is caused by protozoan parasites called Plasmodia, belonging to the parasitic phylum *Apicomplexa*. More than 200 species of the genus *Plasmodium* have been identified that are parasitic to reptiles, birds, and mammals (Abdalla *et al*2007).

Four *Plasmodium* species have been well known to cause human malaria, namely, *P. falciparum, P. vivax, P. ovale, and P. malariae.* A fifth one, *P. knowlesi,* has been recently documented to cause human infections in many countries of Southeast Asia(Daneshvar, 2009).

In 2015, 91 countries and areas had ongoing malaria transmission. Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places. Between 2010 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 21% globally. In the same period, malaria mortality rates among populations at risk fell by 29% globally among all age groups, and by 35% among children under five years old. The WHO African region carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths (WHO, 2017).

2.2 Morphology:

The blood-stage parasites of human *Plasmodium* species exhibit differences in their morphology and modify the host erythrocyte differently. These differences can be used to distinguish the four species. *P. falciparum* blood smears are characterized by the presence of young

trophozoites (rings) in the absence of mature trophozoites and schizonts. The ring stages of *P. falciparum* tend to be slightly smaller than the other species and are generally more numerous. Multiply infected erythrocytes and appliqué forms are seen more often in *P. falciparum* than in the other species. The crescent-shaped gametocytes of *P. falciparum* are very distinctive, but tend to only appear late in the infection. The most distinctive features of *P. vivax* are the enlarged infected erythrocytes and the appearance of granules, called 'Schüffner's dots', over the erythrocyte cytoplasm. These granules are manifestation of caveola-vesicle complexes that form on the erythrocyte membrane. The growing trophozoite of *P. vivax* often has an amoeboid appearance and the schizonts can have more than 20 merozoites.*P. ovale* also exhibits Schüffner's dots and an enlarged erythrocyte, making it difficult to distinguish from *P. vivax*. In general, *P. ovale* is a more compact parasite than *P. vivax*. This compactness is most evident in the growing trophozite stage and fewer merozoites are found per schizont. *P. ovale* also has more of a tendency to form elongated host erythrocytes. *P. malariae* is characterized by a compact parasite (all stages) and does not alter the host erythrocyte or cause enlargement. Elongated trophozoites stretching across the erythrocyte, called band forms, are sometimes observed. Schizonts will typically have 8-10 merozoites that are often arranged in a rosette pattern with a clump of pigment in the center(Kawamoto *et al.*, 2002).

2.3 Transmission:

Principal mode of spread of malaria is by the bites of female *Anopheles* mosquito. Of more than 480 species of *Anopheles*, only about 50 species transmit malaria, with every continent having its own species of these mosquitoes.The habits of most of the anopheline mosquitoes have been characterised as anthropophilic, endophagic, and nocturnal with peak biting at midnight. The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs. When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infested mosquito. When this female mosquito bites another man for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim, thus spreading the infection (Carolina and Sanjeev ,2005).

2.3.1 Other modes of transmission:

Rarely malariacan spread by the inoculation of blood from an infected person to a healthy person. In this type of malaria, asexual forms are directly inoculated into the blood and pre-erythrocytic development of the parasite in the liver does not occur. Therefore, this type of malaria has a shorter incubation period and relapses due to persisting exo-erythrocytic forms do not occur(Dodd,2002).

2.3.2 Mother to the growing fetus (Congenital malaria):

Transfer of parasitized red cells from infected mother to the child either transplacentally or during labor can lead to malaria in the newborn, called as congenital malaria. Congenital malaria seems to be rarely reported and

has always been considered to be more frequent in the non-immune population than in the endemic areas. In recent years, however, higher prevalence of congenital malaria ranging from 8% to 33% has been reported from both malaria-endemic and non-endemic areas, including the United States, Europe, India, etc.(McKenzie*et al*,2003). Congenital malaria has been reported due to all four plasmodium species that commonly infect humans, although most cases are reported following *P. falciparum* or *P. vivax* malaria in the mother. In non-endemic countries, *P. malariae* may cause a disproportionately higher number of congenital malaria cases due to its longer persistence in the host. Congenital malaria occurs more often during the first pregnancy(Denise *et al*,2009.)

2.3.3 Transfusion malaria:

Malaria can be transmitted by transfusion of blood from infected donors. First reported in 1911, transfusion malaria is one of the most common transfusion-transmitted infections today . The risk of acquiring transfusion malaria is very low (1 case per 4 million) in non-endemic countries such as the United States,(Dodd,2002).

Following a malaria infection, the individual may remain infective for weeks to months, or even years, in case of *P. malariae* infection. Therefore, those who have suffered from malaria should not donate blood for at least 3 years after becoming asymptomatic, and proven carriers of *P. malariae* should never donate blood. The risk of transmission is higher in transfusion of fresh, whole blood, particularly when the blood has been stored for less than 5 days and the risk is considerably lesser after 2 weeks (peter and martia,2002).

The risk of transmission is extremely low in case of transfusions of plasma, plasma components, or derivatives devoid of intact red cells . It is difficult to identify malarial infection in donated blood specimens. Most donors implicated in transfusion-transmitted malaria are predominantly semi-immune with very low parasite loads and the infectious dose is estimated to be 1 to 10 parasites in a unit of blood. Detection of such low parasitemia is difficult or impossible with the peripheral smear examination or with more sensitive tests such as the antigen or polymerase chain reaction (PCR) . However, presence of high titer antibodies in such individuals offers some scope for identification of malaria in donated blood and the recent development of enzyme linked immunoassays (ELIAs) with improved sensitivity to *P. falciparum* and *P. vivax*, the predominant transfusion threats, has heightened the appeal of serological testing . Although universal serological screening in nonendemic regions is not cost-effective. The development of automated protein microarray-based technology has the potential to further enhance antibody/antigen sensitivity There are reports of successful use of rapid diagnostic tests RDTs in screening donated blood (Abba *et al*, 2014).

2.4 Life cycle:

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses (Laurence*et al* 2002).

The parasite passes through several stages of development such as the sporozoites ; the infectious form injected by the mosquito, merozoites ; the stage invading the erythrocytes, trophozoites ; the form multiplying in erythrocytes, and gametocytes ;sexual stages and all these stages have their own unique shapes and structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages, that help the parasite to evade the immune clearance, while also creating problems for the development of drugs and vaccines (CDC.,2016)).

Mosquitoes are the definitive hosts for the malaria parasites ; the sexual phase of the parasite's life cycle occur inmosquitoes. The sexual phase is called sporogony and results in the development of innumerable infecting forms of the parasite within the mosquito that induce disease in the human host following their injection with the mosquito bite. When the female *Anopheles* draws a blood meal from an individual infected with malaria, the male and female gametocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gametocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle. The

male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. (CDC.,2016)).

Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of about 8–15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium*-infected mosquitoes have a better survival and show an increased rate of blood-feeding, particularly from an infected host(CDC.,2016).

2.5 Pathology and clinical features:

Malaria is an acute febrile illness. In a non-immune individual, symptoms usually appear 10–15 days after the infective mosquito bite. The first symptoms; fever, headache, and chills– may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death.Children with severe malaria frequently develop one or more of the following symptoms;severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. In malaria endemic areas, people may develop partial immunity, allowing asymptomatic infections to occur (WHO, 2010**).**

2.5.1 Fever:

In an endemic area, it is rather unusual to find cases with typical fever pattern. Some patients may not have fever at all and may present with other symptoms . Many present with fever of various patterns – low grade to high grade, with or without chills, intermittent to continuous, or even as cases of prolonged fever. In the initial stages of the illness, fever may be quotidian, with more than one spike per day and this is due to the development of multiple broods of the parasite,as the disease progresses, these broods get synchronized and the fever tends to be more uniform. However in cases of *P. falciparum* malaria and mixed infections, this pattern of multiple spikes may continue(Srinivas, 2015).

The characteristic feature of malaria is fever caused by the release of toxins ,which stimulate the secretion of cytokines from leukocyte and

other cells, in early stages of infection the fever is irregular or continuous (Srinivas,2015)) .

Typical malaria fever attack starts with cold stage (rigor) in which the patient shivers and feels cold ,even though his or her temperature is rising ,a hot stage follows in which the temperature is rises to the maximum, headache is sever, and there are back and joint pains, vomiting, and diarrhea. The final stage is when the patient perspire(sweating) the temperature fall, headache and other pains are relieved and patient feel exhausted (Srinivas, 2015).

2.5.2 Anemia:

Anemia is a common manifestation of all types of malaria. It is more common and poses more problems in pregnancy and children. In falciparum malaria, anemia can develop rapidly due to profound hemolysis. The degree of anemia correlates with parasitemia and schizontemia. It is also associated with high serum bilirubin and creatinine levels. Pregnancy, secondary bacterial infections and bleeding disorders like disseminated intravascular coagulation can aggravate the anemia. Children may have severe anemia even with low parasitemia and in such cases the reticuloendothelial cells exhibit abundant malarial pigments(Srinivas, 2015).

Anemia in malaria is multifactorial. The causes include obligatory destruction of red cells at merogony, accelerated destruction of non-parasitised red cells (major contributor in anemia of severe malaria), bone marrow dysfunction that can persist for weeks, shortened red cell survival and increased splenic clearance. Massive gastrointestinal haemorrhage can also contribute to the anemia of malaria. Patients with anemia can

present with tiredness, prostration, breathlessness or even severe left ventricular failure and pulmonary edema.In pregnancyanemia can cause premature labour, still birth and high perinatal and maternal mortality. Anemia and fever tend to increase the cardiac output and this combination can prove fatal for patients with pre-existing cardiac disease.The importance of anaemia as a cause of death in malaria may well be underestimated because of difficulty in diagnosis, especially where parasitaemia may be low and the clinical picture may be confused with other causes of anaemia. Two clinical presentations predominate; severe acute malaria in which anaemia supervenes, and severe anaemia in patients in whom there have been repeated attacks of malaria. The major mechanisms are those of red cell destruction and decreased red cell production. Potential causes of haemolysis 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(Srinivas, 2015)).

The type of anemia is hemolytic, normocytic, normochromic. The degree of anemia is greater than what could be explained by the destruction of parasitized red cells. In addition, there occurs increased destruction of red cells possibly by autoimmune mechanisms, and decreased erythropoiesis (Paniker, 2007).

2.5.3 Black water fever :

Blackwater fever,also called malarial hemoglobinuria , one of the less common yet most dangerous complications of malaria. It occurs almost exclusively with infection from the parasite*p.falciparum*. Blackwater fever has a high mortality. Its symptoms include a rapid pulse, high fever and chills, extreme prostration, a rapidly developing anemia, and the passage of urine that is black or dark red in color (hence the disease's name). The distinctive color of the urine is due to the presence of large amounts of hemoglobin, released during the extensive destruction of the patient's red blood cells by malarial parasites. Patients frequently develop anemia because of the low numbers of red blood cells. The presence of blood pigments in the blood serum usually produces jaundice early in the course of the disease. Blackwater fever is most prevalent in Africa and Southeast Asia. Individuals with increased susceptibility, such as non immune immigrants or individuals who are chronically exposed to malaria, are classic sufferers from the complication. Blackwater fever seldom appears until a person has had at least four attacks of malaria and has been in an endemic area for six months. Treatment for blackwater fever includes antimalarial drugs, whole-blood transfusions, and complete bed rest, but even with these measures the mortality remains about 25 to 50 percent.(Srinivas, 2015).

2.5.4Splenomegaly:

Splenomegaly is defined as enlargement of the spleen. In the past, splenomegaly was a clinical finding, but in recent years, imaging studies have also helped to assess for or confirm mild splenomegaly. The prognosis for patients with splenomegaly is usually excellent and not substantially different from age-matched controls, but it is impacted by

the underlying disease state rather than the presence of splenomegaly or the post-splenectomy state(David-West ,1990).

Morbidity and mortality in cases of splenomegaly principally stem from associated disease states or surgical procedures, rather than from the splenomegaly itself. The rates for morbidity and mortality are highly variable and relate to the presence or absence of morbidities, hemorrhage, and organ failure. Patients with enlarged spleens are more likely to have splenic rupture from blunt abdominal or low thoracic trauma. These patients are unlikely to undergo non operative management of their splenic injury or splenic salvage maneuvers, because their spleen is abnormal with regard to architecture, capsule tensile strength, and, commonly, hemostatic function(David-West, 1990).

2.5.5Tropical splenomegaly syndrome :

Several reports were published over the last century describing patients from tropical areas with massive splenomegaly. After excluding known causes of splenomegaly, tropical splenomegaly syndrome was defined as a separate entity. This condition was later defined as hyperreactive malarial syndrome (HMS) using clear diagnostic criteria.(peter and martia,2002) . (HMS) is prevalent in native residents of regions where malaria is endemic and visitors to those regions. Patients with HMS have high levels of antibody for *P. falciparum, P. vivax,* or *P.ovale*.(Erhart*et al* 2004).

Chronic antigenic stimulation may be an important factor in the development of HMS . Although the exact mechanism is uncertain, evidence suggests that repeated or chronic exposure to malaria elicits exaggerated stimulation of polyclonal B lymphocytes, leading to

excessive and partially uncontrolled production of immunoglobulin M(IgM) as the initiating event.IgM is polyclonal and is not specific for any particular malarial species. (HMS) has been reported only in people who have resided in or who have visited areas where malaria is endemic (peter and martia,2002).

2.5.6 Cerebral malaria:

Is the most common complication and cause of death in severe *P. falciparum* infection. In falciparum malaria, 10% of all admissions and 80% of deaths are due to the CNS involvement. On the other hand, CNS manifestations are fairly common in malaria and it could be due to not only severe *P. falciparum* infection, but also high-grade fever and antimalarial drugs. Therefore, it is extremely important to differentiate between these so as to avoid unnecessary anxiety and improper treatment. Cerebralmalaria is the most important complication of falciparum malaria. However, its pathophysiology is not completely understood. Erhart *et al* , 2004).

The basic underlying defect seems to be clogging of the cerebral micocirculation by the parasitized red cells. These cells develop knobs on their surface and develop increased cytoadherent properties, as a result of which they tend to adhere to the endothelium of capillaries and venules. This results in sequestration of the parasites in these deeper blood vessels. Also, rosetting of the parasitized and non-parasitized red cells and decreased deformability of the infected red cells further increases the clogging of the microcirculation. (Erhart*et al*, 2004).

It has been observed that the adhesiveness is greater with the mature parasites. Obstruction to the cerebral microcirculation results in hypoxia and increased lactate production due to anaerobic glycolysis. The

parasitic glycolysis may also contribute to lactate production. In patients with cerebral malaria, C.S.F. lactate levels are high and significantly higher in fatal cases than in survivors. The adherent erythrocytes may also interfere with gas and substrate exchange throughout the brain. However, complete obstruction to blood flow is unlikely, since the survivors rarely have any permanent neurological deficit. Vascular permeability is found to be mildly increased, however, no definite evidence of cerebral edema has been found on imaging studies. The mechanism of coma is not clearly known. Increased cerebral anaerobic glycolysis, intereference with neurotransmission by sequestered and highly metabolically active parasites has been blamed. Cytokines induce nitric oxide synthesis in leukocytes, smooth muscle cells, microglia and endothelium and NO is a potent inhibitor of neurotransmission.(Erhart,*et al* 2004).

2.6 Malaria caused by *Plasmodiumfalciparum*

Malaria caused by *P.falciparum* referred to as *falciparum* malaria formerly known as malignant tertian malaria accounting for up to 80% of malaria cases worldwide(Forney *et al*., 2001). *P. falciparum* is most pathogenic of the human malaria species with untreated infections causing sever disease and death, particularly in young children ,pregnant women, and non-immune adults(Forney *et al*., 2003).

The pathogenicity of *p. falciparum* is mainly due to the cytoadherance of *falciparum* parasitized red cells causing the cells to adhere to one another and to wall of capillaries in the brain, heart, spleen, intestine, lungs, and placenta and due to sequestration of parasitized cells in the microcirculation causes congestion, hypoxia, blockage and rupturing of small blood vessels.(Erhart *et al*., 2004).

The most serious and fatal type of malaria is malignant tertian (MT) malaria caused by *P. falciparum*. When not treated promptly and adequately, dangerous complications develop. The term pernicious malaria has been applied to a complex of life-threatening complications that sometimes supervenes in acute *P.falciparum* malaria. These may present in various forms, the most important of which are the cerebral, algid and septicaemic varieties. These occur following heavy parasitisation of red cells. The parasitized red cells become deformed, sticky and adhere on the capillary endothelium in internal organs causing anoxic damage, oedema and inflammatory reaction (Paniker, 2007).

2.7 Laboratory diagnosis:

Diagnosis of malaria involves identification of malaria parasite or its antigens in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors. The different forms of the four malaria species; the different stages of erythrocytic schizogony; the endemicity of different species; the population movements; the inter-relation between the levels of transmission, immunity, parasitemia, and the symptoms; the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues; and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitemia on a diagnostic test (Abba *et al* 2011).

2.7.1Blood films

The microscopic tests involve staining and direct visualization of the parasite under the microscope. For more than hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. The careful examination of a well-prepared and well-stained blood film currently remains the “gold standard” for malaria diagnosis. The most commonly used microscopic tests include the peripheral smear study and the Quantitative Buffy Coat (QBC) test.(Abba *et al* 2014).

2.7.2 Rapid diagnostic methods:

Detection in patient samples of malaria parasite antigens such as histidine rich protein II (HRP-II) or *Plasmodium* lactate dehydrogenase (pLDH) can be performed by rapid, point-of-care tests based on immune chromatographic methods. There are many commercially-available rapid tests such as ParaSight F and OptiMAL.(Forny *et al*.,2003).

Advantages of these tests are that they are quick to perform and have high sensitivity. The disadvantages of the rapid format are the relatively high cost, the inability of some tests to distinguish malaria species, and manufacturing variation. Those based on HRP II detection may give positive results in the convalescent phase of the illness due to the persistence of HRP II in the blood after parasite clearance. **(**Carolina and Sanjeev , 2005.).

2.7.3Quantitative buffy coat method(QBC):

This method for identifying the malaria parasite in the peripheral blood. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under an ultraviolet (UV) light. Briefly, blood is collected (from a finger prick) in an haematocrit tube containing acridine orange and anticoagulant. The haematocrit tube is centrifuged at 2000 g for 5 min and immediately examined using a microscope equipped with a UV light . The parasite nuclei fluoresce bright green, and the cytoplasm appears yellow-orange. This test has sensitivity similar to the conventional thick blood film microscopic methods. It is reliable and user-friendly and should be used together with thick blood film microscopic screening. However, QBC requires

specialised instrumentation, has a higher high cost than microscopic methods and is poor at species determination and parasite quantification.

2.7.4 Serological methods

Serological tests for the diagnosis of malaria infection rely on the detection of antibodies against asexual blood stages of the malaria parasite. The first serological test used for the detection of malaria antibodies was the immune fluorescence assay (IFA) . This method uses specific antigen or crude antigen prepared on a slide, coated and kept at –30 c°until use, and quantifies both IgG and IgM antibodies in patient serum samples. Serological tests provide retrospective confirmation of malaria infection or a history of infection, and are useful in epidemiology surveys and the screening of blood collected for blood banks. Nevertheless, the utility of serological methods for the diagnosis of acute malaria infection is limited owing to the delay in antibodies development, lack of species confirmation and the need for a fluorescence (UV) microscope (Abba *et al* 2011).

2.7.5 The polymerase chain reaction(PCR):

Allows the specific amplification of a selected region of the malarial genome. This technique is highly specific and sensitive and permits genotyping. Furthermore, PCR using single nucleotide polymorphism (SNP) analysis allows the detection of drug resistant parasites and mixed infections However, PCR is expensive and requires a sophisticated laboratory manned with well-trained staff.(Rich and Ayala,2006.)

2.8 Treatment:

Malaria is an entirely preventable and treatable disease. The primary objective of treatment is to ensure a rapid and complete elimination of the *Plasmodium* parasite from the patient's blood in order to prevent progression of uncomplicated malaria to severe disease or death, and to chronic infection that leads to malaria-related anemia. From a public health perspective, treatment is meant to reduce transmission of the infection to others, by reducing the infectious reservoir and by preventing the emergence and spread of resistance to antimalarial medicines (srinivas 2015).

2.8.1Treatment of *P.falciparum* infections:

World health organization recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by the *P. falciparum* parasite. By combining two active ingredients with different mechanisms of action, ACTs are the most effective antimalarial medicines available today (WHO, 2010).

The choice of ACT should be based on the results of therapeutic efficacy studies against local strains of *P. falciparum* malaria (WHO,2016).

In low transmission areas, a single low dose of primaquine should be added to the antimalarial treatment in order to reduce transmission of the infection(WHO2016).

2.9 Prevention and control:

Malaria is preventable and curable and increased efforts are dramatically reducing the malaria burden in many places. National malaria control programs need to take special measures to protect these population groups from malaria infection, taking into consideration their specific circumstances (WMR,2015).

There are many factors affecting transmission distribution and abundance of the mosquito vector; these include; temperature and extent of water for larval breeding, seasonal fluctuation of mosquito populations, vectorial capacity of the common vector species and duration of conditions suitable for mosquito survival (WMR,2015). Vector control is the main way to prevent and reduce malaria transmission. Two forms of vector control are effective in a wide range of circumstances: insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS)(Berkins and Bell,2008).

2.10 Malaria and white blood cells:

White blood cells (WBCs) are one of the numbers of different cells that play a part in the body's defenses and give immunity against disease . Their numbers may be reduced (leucopenia) by starvation, pernicious anemia, and certain infections, such as typhoid and malaria. An increase in their numbers (leukocytosis) is a reaction to normal events such as digestion, exertion, and pregnancy, and to abnormal ones such as loss of blood, cancer, and most infections. The mean WBCc was 7.5× 10(9)/L, which is within the range of 4.0–11 × 10(9)/L accepted worldwide(Jadhav *etal*, 2003**.)**

The WBC counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis(Leukocytosis is typically reported in a fraction of cases malaria and may be associated with concurrent infections and/or poor prognosis (McKenzie*et al*, 2003).

Chapter three

3-Material and methods

3.1Study design:

It is cross-sectional study.

2.2 Study area:

This study was conducted in Bashaier hospital Khartoum state during the period between March 2017to May 2018.

2.3 Study population:

Participant of the studycomprised malaria patients of both sex (male and female) who diagnosed recently having malaria

2.4 Data collection:

A well structed questioner was designed used to collect data from

participant in study (appendix2).

2.5 Sample size:

Blood samples were collected from 250 patients came to Bashaier hospitals in Khartoum state during the period of the study.

2.5.1 Sample inclusion criteria:

Patient that having malaria positive were included in this study.

2.5.2 Sample exclusion criteria:

Patients that have bacterial or virus infection which effect on the level of WBCs count . and patients that elder than 60 years old were excluded

from this study.

2.6 Sampling collection:

Three ml of venous blood were collected from diagnosed malaria patients in EDTA container using disposable syringes after disinfecting the skin with 70% alcohol.

2.7 Methodology:

2.7.1Thick blood film examination:

Thick blood film made for count the parasite against 200 WBCs microscopically.Thick blood films prepared on clean slides then stained by gGiemsa stain(10% concentration) for ten minutes, then washed by clean water and dried by air , asexual stage of P. falciparum parasites were counted against 200 WBCs using binocular light microscope .(Olympus CH20) with oil immersion lens (100X)

3.7.2 TWBCs counting:

White blood cells (WBCs) were counted using Sysmex KX-21N (automated hematology analyzer).

3.7.2.1 Principle of automated hematological analyzer system (Sysmex):

Blood sample were aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both sides

of the aperture, there are the electrodes between which flows direct current.

Blood cells which are suspended in the diluted sample pass the aperture, causing direct resistance to change between the electrodes. As direct current resistance changes, the blood cell size was detected as electrode pulses. Blood cell count was calculated by counting the pulses, and a histogram of blood cell sizes was blotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data . Reagents needed were checked for expiry date before use, the samples were analyzed according to the protocol recommended(Sysmex Corporation, 2004).

2.7.3 Calculation of parasitemia:

Parasitemia were calculated by using actual number of WBCs and assumed WBCs by following formula:

Parasite density by actual WBCs

$$= \frac{numberofparasites \times twbcs}{200}$$

Parasite density by assumed WBCs**:**

3.8 Data analysis:

Date was analyzed using SPSS program. The one sample T- test was used for difference in proportion. P. value < 0.05 was taken as cut of value for 95% statistical significance .

3.9 Ethical consideration:

The ethicalapproval was obtained for this study by the Committee of college of medical laboratory science of Sudan university of science and technology.Informed consent was taken from each patient before data collection and for children from their guardian. No information on the patients would presented in this study except the age. Blood would been taken by an experience person on the hospitals ,and under the supervision of the responsible doctor

Chapter four

Result

4.1 General characteristics of the study population:

This study was conducted on 250 subject. From them 135 (54%) were females and 115 (46%) were males (figure 4.1). The age of subject were ranged between 1-67 years old with mean age 19 years old SD 15± the age groups were divided into (1-15, 16-30, 31-45,and 46-67) years old. The frequency of each group as follow 125 (50%), 88(35.2%), 27(10.8%)and 10 (4%) respectively (table 4.1).

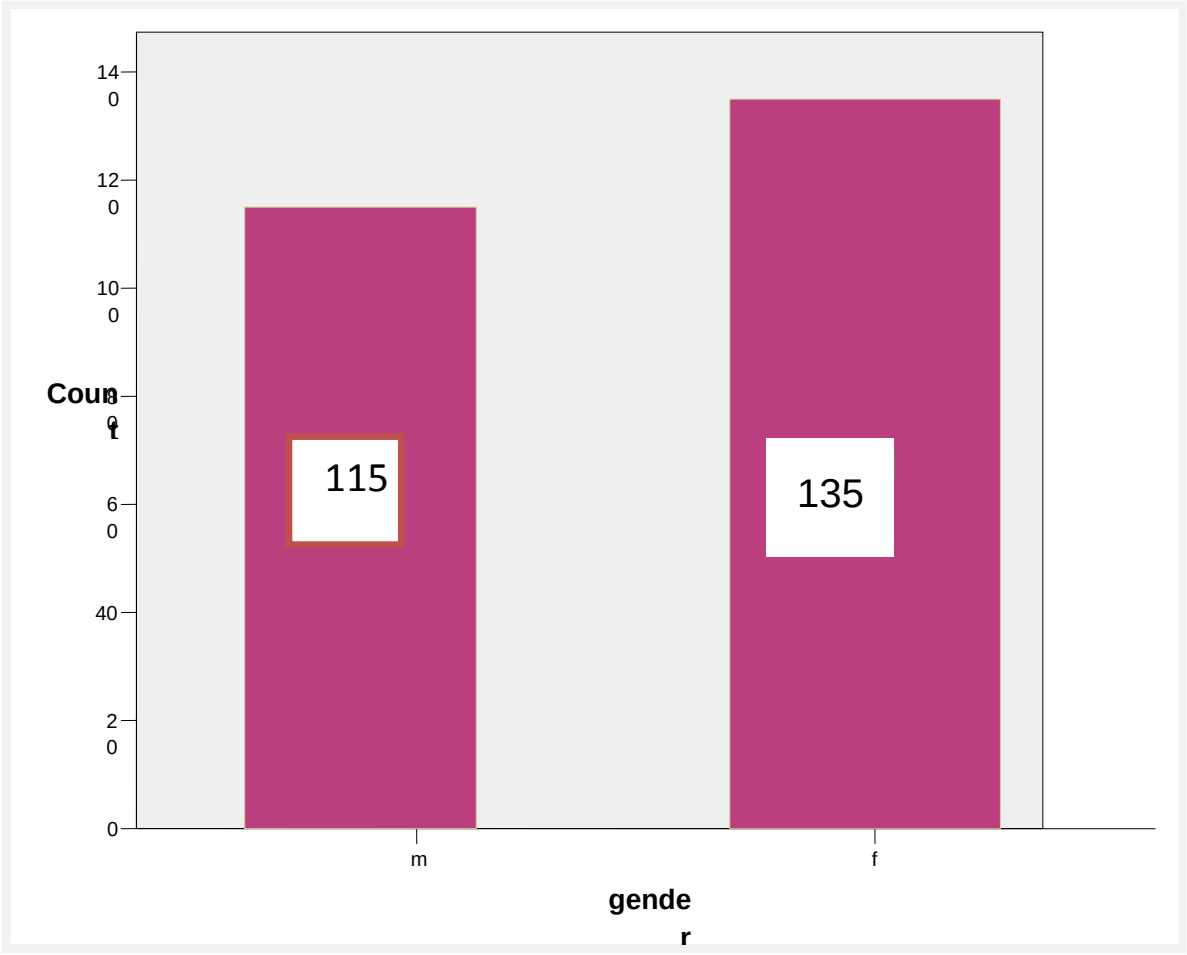

Figure (4.1): Distribution of respondents according to the gender.

Table (4.2): Over all frequency of age groups :

Age group	Frequency	Percentage %
1-15	**125**	**50**
16-30	**88**	**35.2**
31-45	**27**	**10.8**
46-60	**10**	**4.0**
Total	**250**	**100**

4.2 Estimation of white blood cells among study population:

The study show that the mean of WBCs count among malaria patient was (5570.88) ranged (9800-2000), with (SD) (1659).

Table (4.2): The mean and (SD) of WBCs count among malaria patient:

Result	Mean	Max	Min	SD
TWBCs	5570.88	9800	2000	**1659**

4.3 Relationship between parasite density and age groups:

The study show that, the highest mean and SD of parasitemia according to age groups found in age group 1 – 15 years old (12580±8161). Table 4.2).

Table (4.3):Parasite density among age groups:

Age group	parasite density	SD
1-15	12580	**8161**
16-30	12428	**6457**
31-45	102665	**6684**
46-60	11775	**6684**

4.4The comparison of parasite density between actual and assumed WBCs:

The revealed that the mean of parasite density calculated by actual and assumed WBCswere (11346.7) and (16092.6) respectively .difference between actual and assumed parasite density was detected

statistically significant at P-value.see table (4.4)

Table (4.4) Estimation of parasite density using actual and assumed WBCs:

Result	Mean	Max	Min
Actual density	11346.745	12279.44	**10414**
Assumed density	16092.640	17175.46	**15009**

Chapter five

5-Discussion ,Conclusion and Recommendation

5.1Discussion:

This study was conducted in 250 subject,out of them 115 (46%) were males and 135 (54%) were females. The result of the study show that the. These results is consistence with the study carried out at Sudan by Bilal *et al* (2013) . Who showed that the mean (SD) of WBCs was 6.2 9(2.9) cells $\times 10^9$/l. The geometric mean (SD) of the parasite count using the assumed was significantly higher than that estimated using actual WBC count 7345.76 (31038.56) vs.5965(28061.57) ring /µl.The present study show that the mean (SD) of actual WBCs were 5.5 (1.6) cells $\times 10^9$/l. and the geometric mean (SD) of parasite count using the actual WBCs count is(11346.7) lower than that counted using assumed WBCs count(16092.6) this result is consistence in study remembered above. But we enrolled age from 1 to 60 years old and the study of Bilal *et al.* (2013) enrolled in children.

Our results were disagreed to study done by Haggaz *et al*. (2014) in mean of actual WBC, who was reported slightly higher (11.3 c/cumm) and lower in assumed (16.0 c/cumm). This may be due to population in the two study were different, their target were pregnant women.

Other study done by Adu-Gyasi *et al.* (2012) in Ghana in pregnant women used different assumed WBC count and actual WBC count and their result was agreed to us in the assumed WBC were give higher WBC density and the actual WBC count.

Study done by Laman *et al.* (2014) in Papua New Guinea was reported no significant differences between parasite density when using actual and assumed WBC count and we dis agreed with this study. We think this due Laman *et al.* (2014) did this comparison in children and we used different aged.

5.2Conclusion:

The result of the study show that the uses of actual TWBC counts in count malaria parasitic density is more appropriate than the assumed TWBCs.

5.3 Recommendation:

Further studies should be done on large numbers of respondents to estimate the TWBCs during malaria infection.

This study were recommended by review the estimated actual white blood cells count instead of uses assumed WBCs count for calculate ion of parasite density.

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Appendix(1)

Devices:

Analyzer hematology device Sysmex KX-21N.

Binocular light microscope (Olympus CH20).

Reagents:

Giemsa stain

Buffered water, pH 7.1–7.2

Appendix (2)

Sudan University of Science and Technology

Faculty of medical laboratory

MSC in parasitology and medical entomology

Questionner

Questionner about

Malaria Parasite Density Estimation Using Actual And Assumed White Blood Cells Count In Sudan Khartoum State

Patient No Date 2017/ /

Name:…….

Gender: male ……female ……

Age:……years

Laboratory result:

Number of asexual stages of parasite against 200 WBCs ……

TWBCs…….

Parasite density……..