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Sudan University of Science and Technology

College of Graduate Studies

Effect of Malaria Parasite on Complete Blood Counts- Khartoum State

تأثير طفيل الملاريا على تعداد الدم الكلي- ولاية الخرطوم

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

By

Marwa Mohamed Elhag Saeed Mustafa

B.Sc. (Honours) in Medical Laboratory Science (Parasitology and Medical Entomology)- Sudan University of Science and Technology- 2014

Supervisor

Dr. Tayseer Elamin Mohamed

Assistant Professorof Parasitology and Medical Entomology- Sudan University of Science and Technology

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الآية

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

۞ وَمَا مِن دَآبَّةٍ فِي ٱلْأَرْضِ إِلَّا عَلَى ٱللَّهِ رِزْقُهَا وَيَعْلَمُ مُسْتَقَرَّهَا وَمُسْتَوْدَعَهَا ۚ كُلٌّ فِي كِتَٰبٍ مُّبِينٍ ٦

صدق الله العظيم

سورة هود

الآية: ٦

Dedication

To my father,

To my mother,

To my brothers and sisters,

To my teachers,

To my friends,

To my colleagues,

To my students

To everyone who supported me

I dedicate this work

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Thanks firstly and finally to Allah Almighty for blessing and giving me the power to complete this research.

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Abstract

This cross-sectional study was conducted in Khartoum state during the period from February to November 2016. The aim of this study was to determine the effect of malaria parasite on complete blood counts.

Two hundred and seven malaria-infected subjects were included in this study with the age ranged between 1-83 years old with a mean age of 21± 16 years old. Out of these, 103 (49.8%) being males and 104 (50.2%) females. Blood samples were taken from all subjects. Epidemiological data were obtained and recorded. All samples were examined to detect *Plasmodium* species infection in stained thick and thin blood films. Hematological parameters were obtained by analyzing EDTA blood samples, using automated hematological analyzer system (Sysmex-KX2IN).

The study showed that the overall prevalence of *P.falciparum*, *P.vivax* and mixed infections between *P.falciparum* and *P.vivax*, *P.falciparum* and *P.malariae* and *P.vivax* and *P.malariae* were 175 (84.5%), 13(6.3%), 17(8.2%), 1(0.5%) and 1(0.5%) respectively. The study showed that the prevalence of *Plasmodium* infections was highest (44.4 %) among the age group (0-15) years old; most of them were *P.falciparum* infections (38.2 %).

When the results were analyzed, the study indicated that *P.falciparum* infection cause significant hematological changes with platelets count in different levels of parasitemia, also mixed infection cause significant hematological changes with total white blood cells count in different levels of parasitemia (p-value < 0.05).

مستخلص الدراسة

أجريت هذه الدراسة المستعرضة في ولاية الخرطوم خلال الفترة من فبراير إلى نوفمبر ٢٠١٦. وكان الهدف من هذه الدراسة هو تحديد تأثير طفيل الملاريا على تعداد الدم الكلي.

تضمنت الدراسة ٢٠٧ شخص مصاب بالملاريا تراوحت أعمارهم بين ١-٨٣ سنة، وكان متوسط العمر ٢١ ± ١٦ سنة. منهم ١٠٣ (٤٩,٨%) ذكور و١٠٤ (٥٠,٢%) إناث. أخذت عينات الدم من الأشخاص الخاضعين لهذه الدراسة. تم الحصول على البيانات الوبائية وتم تسجيلها. تم الكشف عن عدوى الملاريا وأنواعها عن طريق المسحة المصبوغة الثقيلة والخفيفة. وتم الحصول على معدلات تعداد الدم الكلي من عينات الدم بواسطة جهاز قياس الدم الآلي (Sysmex-KX21N).

أظهرت الدراسة أن انتشار المتصورة المنجلية (*P.falciparum*)، المتصورة النشيطة الدقيقة (*P.vivax*)، العدوى المشتركة بالمتصورة المنجلية و المتصورة النشيطة الدقيقة((*P.falciparum and P.vivax*)، المتصورة المنجلية والمتصورة الملارية (*P.falciparum* and *P.malariae*)، والمتصورة النشيطة الدقيقة و الملارية (*P.vivax* and *P.malariae*) كان ١٧٥ (٨٤,٥%)، ١٣ (٦,٣%)، و١٧ (٨,٢%) ، ١(٠,٥%) و ١(٠,٥%) على التوالي. أظهرت الدراسة أن الانتشار عالٍ جداً بنسبة ٤٤,٤ % في الفئة العمرية (١٥-٠) سنة، ٣٨,٢% منها كانت عدوى بالمتصورة المنجلية.

عند تحليل النتائج، بينت الدراسة أن العدوى بالمتصورة المنجلية تؤثر على تعداد خلايا التخثر في المستويات المختلفة للطفيل في الدم، أيضاً العدوى المشتركة تؤثر على تعداد خلايا الدم البيضاء في المستويات المختلفة للطفيل في الدم (القيمة المعنوية < ٠,٠٥).

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Chapter one

Introduction and literature review

1.1 Introduction:

Malaria has been one of the most prominent and ancient diseases which has been profiled and studied. It has been one of the greatest burdens to mankind, with a mortality rate that is unmatched by any other modern disease other than tuberculosis. This dreadful disease, caused by four different agents (*Plasmodium falciparum, Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*) of the same genus, is a major health problem in most of the countries in the tropics (Sudhakar and Subramani, 2007). Over the years, as per statistical records, it has been estimated that there may be three hundred to five hundred million new infections and one to three million infection related deaths annually caused by malaria, and it has also been found that more than 90% of these deaths occur in the regions in and around Sub-Saharan Africa (Sudhakar and Subramani, 2007). Malaria kills in 1 year what AIDS killed in 15 years. It accounts for 2.6 percent of the total disease burden of the world. It is responsible for the loss of more than 35 million disability-adjusted life-years each year (Sudhakar and Subramani, 2007). The clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other febrile illnesses common in tropical regions (Jairajpuri *et al*., 2014). This impairs diagnostic specificity and often promotes the indiscriminate use of antimalarials (Jairajpuri *et al*., 2014). As parasites of the blood for the majority of their complex life cycle, they expectedly induce haematological alterations (Jairajpuri *et al*., 2014).

Haematological abnormalities are considered a hallmark of malaria and statistical analyses have shown that many of these haematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria

(Jairajpuri *et al*., 2014).The haematological abnormalities that have been reported to invariably accompany infection with malaria include anaemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC) (Facer,1994 and Perrine *et al*., 1982). There have also been reports of leucopenia and leucocytosis (Murphy and Oldfeild, 1996). These and many other studies concluded that haematological abnormalities can provide a diagnostic clue in a patient with acute febrile illness in endemic areas, thus increasing the probability of correctly diagnosing malaria and enhancing prompt initiation of treatment.

1.2 Literature review

Malaria is caused by a protozoan parasite of the genus *Plasmodium*. The most common species are *P.falciparum*, *P.ovale*, *P.vivax* and *P.malariae* (Svenson *et al*., 1995). The most severe forms of malaria are caused by *P.falciparum*, with other species rarely producing serious complications, debilitating relapses, and even death (Svenson *et al*., 1995). Malaria is an important cause of death and illness, especially in tropical countries (Trampuz *et al*., 2003). Malaria affects more than 2400 million people, over 40% of the world's population, in more than 100 countries in the tropics from South America to the Indian peninsula (Sudhakar and Subramani, 2007). The tropics provide ideal breeding and living conditions for the *Anopheles* mosquito, and hence this distribution (Sudhakar and Subramani, 2007). The World Health Organization estimates that in 2015 malaria caused 214 million clinical episodes, and 438,000 deaths (WHO, 2015).

1.3 Transmission:

Principal mode of spread of malaria is by the bites of female *Anopheles* (from Greek hurtful, harmful) mosquito. Of more than 480 species of *Anopheles*, only about 50 species transmit malaria, with every continent having its own species of

these mosquitoes (Srinivas, 2015). The habits of most of the *Anopheline* mosquitoes have been characterized as anthropophilic (prefer human blood meal), endophagic (bite indoors), and nocturnal (bite at night) with peak biting at midnight, between 11 pm and 2 am (CDC, 2015). The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs (CDC, 2015). The mosquitoes find their host by seeking visual, thermal, and olfactory stimuli and of these; carbon dioxide, lactic acid, skin temperature, and moisture are more important mosquito attractants (CDC, 2015). Depending on the strength of these stimuli, the attractiveness of different persons varies, with adults, men, and larger persons being more attractive than others (Carolina and Sanjeev, 2005 and Mark, 1998).

Other modes of transmission:

Rarely malaria can 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 (Srinivas, 2015). Therefore, this type of malaria has a shorter incubation period and relapses due to persisting exo-erythrocytic forms do not occur (Srinivas, 2015).

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 in malaria in the newborn, called as congenital malaria (Neena *et al*., 2007).

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 (Srinivas, 2015).

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 (Srinivas, 2015).

Needle stick injury: Cases of malaria transmission through needle-stick injuries, accidentally among health care professionals (some even fatal) or due to needle sharing among drug addicts, have also been reported (Chauhan *et al*., 2009, Slinger *et al*., 2001 and Weir, 1997).

1.4 Life cycle:

When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood (Srinivas, 2015). 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 (Srinivas, 2015). 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 (Srinivas, 2015).

The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood (CDC, 2016). In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites "merozoites" that continue the cycle by invading other red cells. The blood stage parasites are those that cause the symptoms of malaria (CDC, 2016). When certain forms of blood stage parasites "gametocytes" are picked up by a female *Anopheles* mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito (CDC, 2016). After 10-18 days, the parasites are found as "sporozoites" in the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another human, the sporozoites are injected with the mosquito's saliva and

start another human infection when they parasitize the liver cells (CDC, 2016). Thus, the mosquito carries the disease from one human to another (acting as a "vector"). Differently from the human host, the mosquito vector does not suffer from the presence of the parasites. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host (CDC, 2016). Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. Note that in *P.vivax* and *P.ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later. After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (figure 1.1) (CDC, 2016).

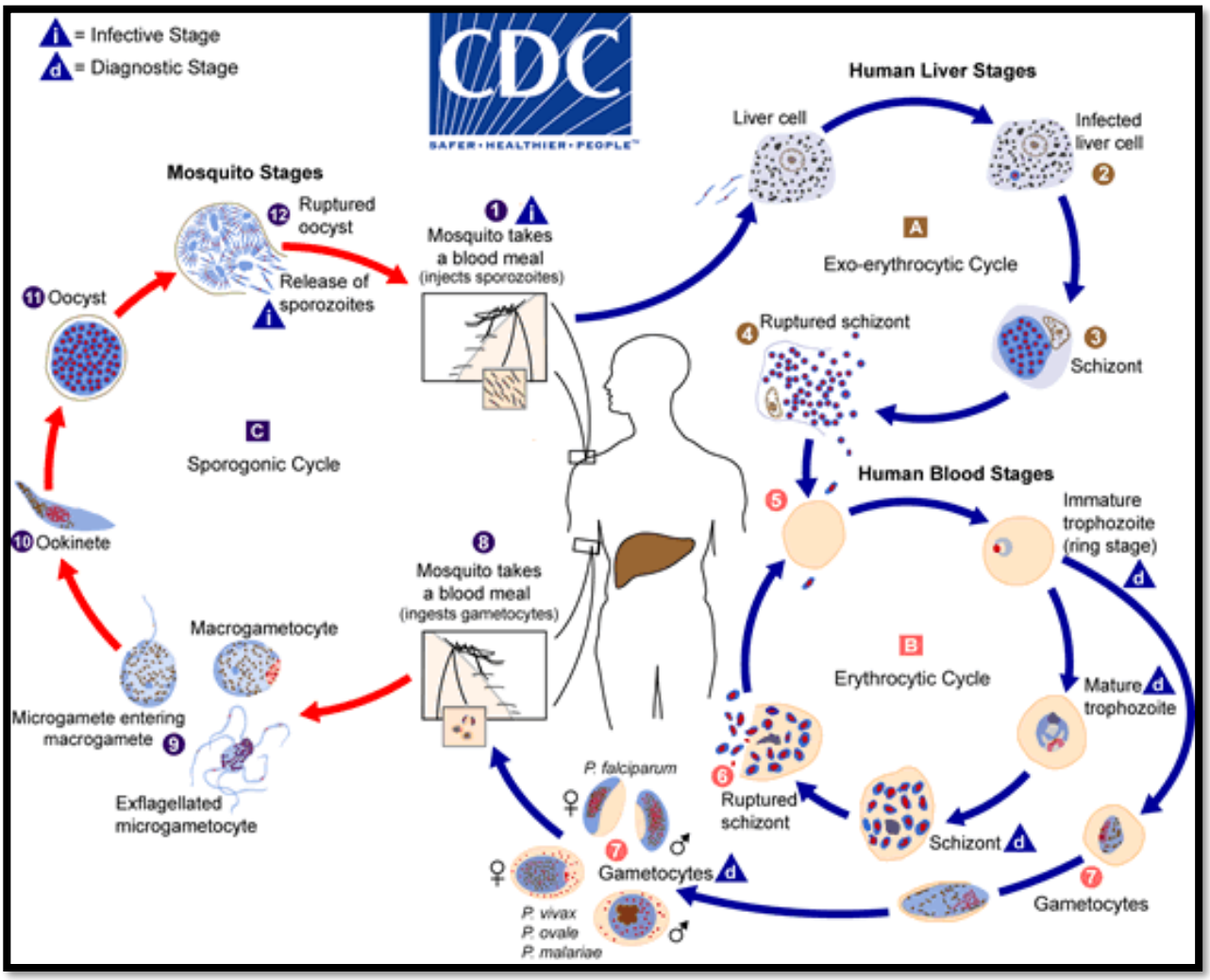

Figure (1.1): Malaria life cycle (CDC, 2016).

1.5 Pathogenesis and clinical picture:

The incubation period varies usually from 8 to 40 days, being shortest in *P.falciparum* and longest in *P.malariae* infections. The average incubation periods are 8-11 days for *P.falciparum*, 10 to 12 days for *P.vivax* and *P.ovale* and 18 to 40 days for quartan malaria. However, very much longer incubation periods, up to 9 months have been recorded with some strains of *P.vivax* (*P.vivax hibernans*) (Paniker, 2007).

The incubation period is to be distinguished from the prepatent period, which is the interval between the entry of the parasites into the host and the time when they first become detectable in blood (Paniker, 2007). The minimum level of parasitaemia for their microscopic detection is called the microscopic threshold. This is about 20 to 25 parasites per cu/mm (Paniker, 2007). Clinical disease develops only later,

when after a number of further cycles of multiplication, the level of parasitaemia rises high enough to cause fever, the so-called fever threshold or pyrogenic density (Paniker, 2007). The first clinical illness marking the end of the incubation period is called the primary attack. The typical picture of malaria consists of periodic bouts of fever with rigor, followed by anemia and splenomegaly (Paniker, 2007). True rigor is typically present in *P.vivax* malaria and is less common in *P.falciparum* infection. The febrile paroxysm comprises three successive stages (Paniker, 2007). In the cold stage, lasting for 15 to 60 minutes, the patient experiences intense cold and uncontrollable shivering. This is followed by the hot stage, lasting for 2 to 6 hours, when the patient feels intensely hot (Paniker, 2007). The fever mounts to 41°C or higher. Severe headache, nausea and vomiting are common. Afterwards, comes the sweating stage, when the patient is drenched in profuse sweat. The temperature drops rapidly and the patient usually falls into deep sleep, to wake up refreshed (Paniker, 2007). The paroxysm usually begins in the early afternoon and lasts for 8 to 12 hours. The periodicity of the attack varies with the species of the infecting parasite (Paniker, 2007). The periodicity is approximately 48 hours in tertian and 72 hours in quartan malaria. Quotidian periodicity, with the fever occurring at 24 hour intervals may be due to two broods of tertian parasites maturing on successive days, or due to mixed infection (Paniker, 2007). Regular periodicity is seldom seen in the primary attack, but is established usually only after a few days of continuous, remittent or intermittent fever (Paniker, 2007). All clinical manifestations in malaria are due to the products of erythrocytic schizogony and the host's reactions to them. The exo-erythrocytic liver cycle and gametogony do not appear to contribute to clinical illness (Paniker, 2007). The febrile paroxysms follow the completion of erythrocytic schizogony, when the mature schizont ruptures, releasing red cell fragments, merozoites, malarial pigment and other parasitic debris (Paniker, 2007). Macrophages and

polymorphs phagocytose these and release large quantities of endogenous pyrogens, leading to elevation of temperature (Paniker, 2007). Cytokines such as tumor necrosis factor (TNF) and interleukin-l (IL-1) may play a pivotal role in the pathogenesis of malarial fever (Paniker, 2007).

Recrudescence and relapse:

After a number of paroxysms, the primary attack subsides with the development of partial immunity in the host. This is followed by a period of latency during which there is no clinical illness or sometimes even parasitaemia (Paniker, 2007). The parasites are not, however, eliminated at this stage, but persist in some erythrocytes, though the level of parasitaemia is below the fever threshold, or sometimes even below the microscopic threshold. Erythrocytic schizogony continues in the body at low levels and gradually the numbers of parasites build up to cross the fever threshold. Fresh malarial attacks then develop (Paniker, 2007). These new malarial attacks that appear after a period of latency usually within eight weeks after the culmination of the primary attack and resulting from persistence of the erythrocytic cycle of the parasites are called recrudescences (Paniker, 2007). Recrudescence may be due to waning immunity of the host or possibly to antigenic variations in the parasite (Paniker, 2007). There may be several such recrudescences, which are generally milder than the primary attack. After a varying number of such attacks, the infection is eliminated in *P.falciparum* and *P.malariae* infections (Paniker, 2007). In *P.vivax* and *P.ovale* infections the parasites may survive for long periods in a dormant exoerythrocytic stage as hypnozoites in liver cells (Paniker, 2007). Reactivation of hypnozoites leads to initiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria caused by the dormant exoerythrocytic forms being reactivated after long periods, usually from 24 weeks to 5 years after the primary attack are called relapses (Paniker, 2007). The term recurrence has been used to refer to both

recrudescence and relapse, and so carries no specific meaning. Several factors including stress, intercurrent infection, pregnancy and alcoholism have been proposed as precipitating causes for recurrences (Paniker, 2007).

Malignant tertian malaria:

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). Cerebral malaria is characterized by hyperpyrexia, coma and paralysis. Algid malaria resembles surgical shock, with cold clammy skin, peripheral circulatory failure and profound hypotension (Paniker, 2007). Gastrointestinal symptoms such as vomiting, dysenteric or choleraic diarrhoea may occur. Some cases develop severe hiccup, with profuse bilious vomiting, a condition formerly called bilious remittent fever (Paniker, 2007). In septicaemic malaria, characterized by a high degree of prostration, there is high continuous fever with involvement of various organs. Acute renal failure and acute pulmonary oedema are other serious complications (Paniker, 2007).

Blackwater fever:

A syndrome called black water fever (malarial hemoglobinuria) is sometimes seen in *P.falciparum* malaria, particularly in patients who have experienced repeated infections and inadequate treatment with quinine (Paniker, 2007). Patients with Glucose 6 phosphate dehydrogenase deficiency may develop this condition after

taking oxidant drugs, even in the absence of malaria (Paniker, 2007). Clinical manifestations include bilious vomiting and prostration, with passage of dark red or blackish urine (black water). The pathogenesis is believed to be massive intravascular hemolysis caused by antierythrocyte autoantibodies, leading to hemoglobinaemia and hemoglobinuria (Paniker, 2007).

Anemia:

Anemia occurs in all types of malaria, but is most pronounced in *falciparum* infections. 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).

Splenomegaly:

The spleen is invariably affected, being always enlarged in malaria. The initial change is congestion, leading to a soft enlargement. Later, it becomes dark due to accumulated malarial pigment (Paniker, 2007). Diffuse cellular hyperplasia, dilated sinusoids and accumulation of macrophages accentuate the enlargement of spleen, which become shard due to fibrosis (Paniker, 2007).

Tropical splenomegaly syndrome:

Tropical splenomegaly syndrome (TSS) also known as hyper-reactive malarial splenomegaly (HMS) is a chronic benign condition seen in some adults in endemic areas, mainly tropical Africa, New Guinea and Vietnam. This results from an abnormal immunological response to malaria and is characterized by enormous splenomegaly, high titres of circulating anti-malaria antibody and absence of malaria parasites in peripheral blood smears. Hyperimmunoglobulinaemia (IgM, but not IgG), cryo-globulinaemia, reduced C3 and presence of rheumatoid factor without arthritis are other features (Paniker, 2007). A normocytic normochromic

anaemia is present, not responding to hematinics or anthelmentics. TSS differs from various other types of splenomegalies seen in the tropics in its response to anti-malarial treatment, and histological changes in spleen (dilated sinusoids lined with reticulum cells showing erythro-phagocytosis, lymphocytic infiltration of pulp) and liver (marked sinusoidal infiltration with lymphocytes). The liver is also congested, enlarged and pigmented. Numerous pigment-laden Kupffer cells dot the liver. Changes are also seen in bone marrow, kidney and adrenals (Paniker, 2007).

Cerebral malaria:

In cerebral malaria, lesions occur in the central nervous system. These consist of congestion of the meninges and brain, occlusion of capillaries in brain, numerous petechial perivascular hemorrhages, and necrotic lesions in mid zonal brain tissue, with peripheral glial reaction (malarial granuloma) around occluded blood vessels (Paniker, 2007).

1.6 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 (Srinivas, 2015).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 (Srinivas, 2015). The useful 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 (Srinivas, 2015). The simplest and surest test is the time-honoured peripheral smear study for malarial parasites. None of the other newer tests have surpassed the ‘gold standard’ peripheral smear study (Srinivas, 2015). Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. Thick smears are 20-40 times more sensitive than thin smears for screening of *Plasmodium* parasites, with a detection limit of 10–50 trophozoites/μl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes (Srinivas, 2015). The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear (Srinivas, 2015). The test takes about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentioned above. Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smear, which has a sensitivity of 90% (Srinivas, 2015). The level of parasitemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood. In non-*falciparum* malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (> 50%) in *P.falciparum* malaria. In nonimmune individuals, hyperparasitemia (> 5% parasitemia or > 250,000 parasites/μl) is generally associated with severe disease

(Srinivas, 2015). The smear can be prepared from blood collected by vein puncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, ethmoid sinus via the nose or through fontanelle in young children can be used (Srinivas, 2015). Many of the new technologies for malaria diagnosis incorporate immunochoromatographic procedure, where conjugated monoclonal antibodies are the key reagents. Currently many rapid diagnostic tests (RDTs) are widely used for the diagnosis of malaria. These RDTs are simple lateral-flow immunochromatographic tests that detect parasite specific antigens released from red blood cells. Two of the tests, the ICT Malaria Pf/Pv and ParaSight-F detect histidine rich protein-2 (HRP-2), a protein produced by asexual stages and young gametocyte of *P.falciparum*. The third test OptiMAL detects *Plasmodium* lactate dehydrogenase (PLDH), a marker protein for the intra-erythrocytic form of the malaria parasite. HRP-2 is an abundant protein produced by all blood stages of *P.falciparum.* Also, there is insufficient data available to determine the ability of this test to detect the 2 less common species of malaria, *P.ovale* and *P.malariae* (Verma *et al*., 2013). Therefore, all negative RDTs must be followed by microscopy to confirm the result (CDC, 2014). Although the rapid diagnostic assays offer a number of attributes that make them attractive for use in the developing world (minimally trained personnel find them easy to use, no equipment is required, and samples can be read with the naked eye), they cannot quantify the level of parasitemia or malarial species, they aren't reliable in the presence of low-level (and occasionally even very-high-level) parasitemia, they remain positive for 7 to 14 days after treatment (CDC, 2014). Alternative microscopic methods have been tried, including faster methods of preparation, dark-field microscopy, and stains like benzothiocarboxypurine,

acridine orange and rhodamine-123. Acridine orange has been tried as a direct staining technique, with concentration methods such as thick blood film or the centrifugal quantitative buffy coat system and with excitation filter in the Kawamoto technique. Inability to easily differentiate the *Plasmodium* species, requirements of expensive equipment, supplies and special training as well as the high cost limit the use of these methods (Srinivas, 2015).

1.7 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 (WHO, 2016b).

Treatment 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, 2016b). WHO currently recommends 5 ACTs for use against *P.falciparum* malaria. The choice of ACT should be based on the results of therapeutic efficacy studies against local strains of *P.falciparum* malaria (WHO, 2016b). 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. Testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency is not required, as a single low dose of primaquine is both effective in

blocking transmission at this low dose and unlikely to cause serious toxicity in individuals with any of the G6PD-deficiency variants (WHO, 2016b).

Treatment of *P.vivax* infections:

P.vivax infections should be treated with chloroquine in areas where this medicine remains effective. In areas where chloroquine-resistant *P.vivax* has been identified, infections should be treated with an ACT, preferably one in which the partner medicine has a long half-life (WHO, 2016b). In order to prevent relapses, primaquine should be added to the treatment; dose and frequency of the administration should be guided by the patient's glucose-6-phosphate dehydrogenase (G6PD) enzyme activity (WHO, 2016b).

Treatment of severe malaria:

Severe malaria should be treated with injectable artesunate (intramuscular or intravenous) for at least 24 hours and followed by a complete 3-days course of an ACT once the patient can tolerate oral medicines. When injectable treatment cannot be given, children under 6 years of age with severe malaria should receive a pre-referral treatment with rectal artesunate before being referred immediately to a health care facility where the full level of care can be provided. In view of the latest development of resistance, it is essential that neither artemisinin-based injectables nor artesunate suppositories be used as mono-therapies, the initial treatment of severe malaria with these medicines needs to be completed with a 3-day course of an ACT (WHO, 2016b).

1.8 Epidemiology:

Malaria is one of the most important public health problems in term of morbidity and mortality, causing more than 200 million cases and 655,000 deaths every year (World Malaria Report, 2011). *P.falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally.

P.vivax has a wider distribution than *P.falciparum*, and predominates in many countries outside of Africa (World Malaria Report, 2011).

According to the latest WHO estimates, released in December 2015, there were 214 million cases of malaria in 2015 and 438 000 deaths. The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%) (WHO, 2015). Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 88% of malaria cases and 90% of malaria deaths. Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region. In that same period, malaria death rates among populations at risk fell by 60% globally among all age groups, and by 65% among children under five years (WHO, 2015). Some population groups are at considerably higher risk of contracting malaria, and developing severe disease, than others. These include infants, children under five years of age, pregnant women and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travelers (WHO, 2015). Young children, pregnant women and non-immune travelers from malaria-free areas are particularly vulnerable to the disease when they become infected (WHO, 2015). Children under five are particularly susceptible to malaria illness, infection and death. In 2015, malaria killed an estimated 306 000 under-fives globally, including 292,000 children in the African Region. Between 2000 and 2015, the mortality rate among children under five fell by 65% worldwide and by 71% in Africa (WHO, 2015).

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 (World Malaria Report, 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 (World Malaria Report, 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). Over the last 15 years, there has been a major increase in coverage of ITNs in sub-Saharan Africa. By 2014, more than half (56%) of the population had access to an ITN, compared to less than 2% in 2000. In 2014, 116 million people globally were protected by indoor residual spraying (IRS), including 50 million people in Africa. About 6% of the populations at risk of malaria in Africa live in households that are protected by IRS (World Malaria Report, 2015).

1.9 Malaria impact on hematological parameters:

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 (Bakhubaira, 2013 and Warimwe *et al*., 2013). Hematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes and thrombocytes (Bakhubaira, 2013 and Warimwe *et al*., 2013). Malaria infected patients tended to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-malaria infected patients (van Wolfswinkel *et al*., 2013 and Adedapo *et al*., 2007). One study showed patients with higher WBCs count

compared with community controls (Ladhani *et al*., 2002). The most common complication during malaria infection is thrombocytopenia (Erhart *et al*., 2004, Moulin *et al*., 2003 and Mahmood and Yasir, 2008). Persons with platelet counts less than 150,000/µl were 12-15 times more likely to have malaria infection than persons with platelet counts more than 150,000/µl (Erhart *et al*., 2004). A previous study found that the ratio of monocytes to lymphocytes correlated with risk of clinical malaria during follow-up (Warimwe *et al*., 2013). Clinical diagnosis is widely used for diagnosis of malaria especially in these areas (Warimwe *et al*., 2013). Fever and other signs and symptoms are known to be sensitive measures of malaria infection but they lack specificity and positive predictive values especially in areas where malaria is less prevalent (Maina *et al*., 2010 and WHO, 2000) and it may be difficult to distinguish the sign and symptom of disease from other viral or bacterial infections (Lathia and Joshi, 2004). Typically, microscopic slide examination of peripheral blood remains the most widely used test and is the gold standard for detecting malaria infection (World Malaria Report, 2010). However, due to it requires technical expertise and is time-consuming in smear examinations. Hematological changes during malaria infection, such as thrombocytopenia and leucocytosis or leucopenia are well recognized. Diagnostic value of these haematological alterations may be easily obtained and useful in people living in malaria endemic areas (Kotepui *et al.*, 2014).

1.10 Malaria in Sudan

Sudan is one of eighteen countries account for 80% of malaria cases with a high burden of morbidity and mortality (World Malaria Report, 2014). However, the national malaria control programme with WHO's support, has reduced the number of malaria cases from more than four million in 2000 to less than one million in 2010 (World Malaria Report, 2014). Between 2001 and 2010, the number of deaths due to malaria reduced by 75% (WHO, 2016). In A study published in 2007, the

incidence was estimated to be about 9 million episodes in 2002 and the number of deaths due to malaria was about 44,000. 2,877,000 children under five years of age had the highest burden (Abdalla *et al*., 2007). According to World Malaria Report in 2015, the high transmission was in south-west of Sudan, lower transmission was in south and south-east of the country (World Malaria Report, 2015).

Figure (1.2) below showed the transmission of malaria in Sudan as reported in World Malaria Report at 2015.

Figure (1.2): Transmission of malaria in Sudan (World Malaria Report, 2015)

Rationale

Approximately, 3.2 billion people were at risk of malaria in 2015. Most malaria cases and deaths occur in sub-Saharan Africa (WHO, 2016a). The effect of malaria parasites on hematological parameters has attracted much interest among scientists. The hematological changes include anemia, thrombocytopenia and disseminated intravascular coagulation (DIC). An understanding of these changes will help in diagnosis and treatment and may also serve to predict and prevent various complications **(**Ranjini *et al*., 2012). Therefore, this study was carried out to assess the effect of malaria parasites on some of the hematological parameters in Khartoum state.

Objectives

- **General objective:**

To study the effect of malaria parasites on complete blood counts in Khartoum state.

- **Specific objectives:**
    - To determine the prevalence of each *Plasmodium* species in the study area.
    - To determine the prevalence of *Plasmodium* species according to age groups.
    - To investigate the effects of *Plasmodium* infections on hemoglobin levels and platelets count, total white blood cells and differential counts.
    - To investigate the effects of *Plasmodium* infections density on hemoglobin levels, total white blood cells and platelets counts.

Chapter two

Materials and methods

2.1 Study design:

It is a across sectional study.

2.2 Study area:

The study was carried out in Khartoum State. Khartoum is the capital of Sudan and is located where the Blue and White Niles merge to form the Nile. The huge, spread-out city is actually made out of three distinct cities (Khartoum, Khartoum North or Bahri, and Omdurman) which are divided by the Nile and its two arms. Khartoum state is quickly growing and ranges between 6 and 7 million, which includes approximately 2 million displaced people from the southern war zone and the drought-affected areas in the west and east. The health situation is depressing and deteriorating. Although the infant mortality rate has declined, it is still quite high, and the ratios of health facilities and personnel have decreased. The area is considered to be endemic for malaria. *P.falciparum* is considered to be the major malaria species in the area, followed by *P.vivax*. Mixed infections of these both *Plasmodium* species may also be present (Eltayeb, 2003).

2.3 Study population:

A total of 207 patients with different ages, who were diagnosed with malaria infection, were selected for the purpose of this study.

2.4 Data collection:

A questionnaire was filled by patients' information which include: name, patient ID number, age and gender (appendix).

2.5 Sample size:

The sample size was calculated using the formula:

$$n = \frac{z^2 pq}{d^2}$$

n = sample size.

z = the normal standard deviate (z = 1.96).

p = the frequency of occurrence of an event.

q = 1-p (the frequency of non occurrence of an event).

d = degree of precision (0.05%).

$$n = \frac{(1.96)^2 \times 0.16 \times (1 - 0.16)}{(0.05)^2} = 207$$

2.6 Samples collection:

Blood was collected using sterile disposable plastic needle EDTA vacoutainer, using aseptic standard non-traumatic vein puncture technique and immediately complete blood count was done (hemoglobin, platelets count, WBCs count and differential), and blood films were prepared.

2.7 Methods:

2.7.1 Preparation and examination of blood films:

To make thick smear, the collected blood was stirred with a corner of slide until an appropriate thickness obtained. To make thin smear, the edge of spreader was placed just in front of the drop of blood. Then it was drawn back until it touches the drop of blood. The blood was allowed to run along the edge of spreader. The spreader was then pushed to the other end of slide with smooth movement. Then the slide was allowed to dry.

Both blood films were stained using Geimsa stain. Thin films were fixed with absolute methanol for 1-2 minutes. Then slides were covered with 10% Geimsa solution for 10 minutes. All slides were washed using clean water and allowed to dry by air. The slides were then examined using light microscope with oil immersion lens. Thick film was used for detection of malaria parasites. For levels of malarial parasitemia, data were grouped into hyperparasitemia (more than 10 parasites/ 1 oil field) considered as (++++), high parasitemia (1–10 parasite/ 1 oil

field) considered as (+++), moderate parasitemia (10–100 parasite/100 oil field) considered as (++) and low parasitemia (less than 10 parasites/ 100 oil field) considered as (+) (Kotepui *et al.,* 2014). The thin films were used for identification of species.

2.7.2 Complete blood count (CBC):

Complete blood count usually indicates wherever there are any abnormalities in blood cells. This was done using Sysmex KX2IN (automated hematology analyzer).

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 (Sysmex Corporation, 2004). Hemoglobin measurement was directly done in the WBCs chamber by spectrophotometer by formation of chromogen cyanomethmoglobin for lytic solution without cyanide measurement of the blank hemoglobin was done for each analytical cycle and during the start-up rising steps (Sysmex Corporation, 2004). Reagents needed were checked for expiry date before use, the samples were analyzed according to the protocol recommended.

Normal values are as follow:

-Hemoglobin concentration:

Males 14.0 - 14.4 g/l.

Females 12.4 –13.5 g/l.

-White blood cell count:

Males: 4.3 - 4.6 10^9/l.

Females: 4.4 - 4.8$10^9$/l.

-Differential white cell count:

Neutrophils: 49.1 - 55.2 (%).

Lymphocytes: 37.4 - 42.1 (%).

Mixed cells count:

Monocytes: 5.3 - 7.5 (%).

Eosinophils: 1.1 - 1.9 (%).

Basophils: 0.02-0.18 (%).

-Platelet count:

206.8- 251.2 x 10^9/l.

(Miri-Dashe *et al*., 2014).

2.8 Data analysis:

Data were analyzed using Statistical Package of Social Sciences (SPSS) version 11.5 computer software.

2.9 Ethical consideration:

Ethical consideration was taken from College of Medical Laboratory Science-Sudan University of Science and Technology. Informed consent was taken from each patient before data collection.

Chapter three

Results

3.1 General characteristics of the study population:

This study was conducted on 207 subjects, from them, 103 (49.8%) were males and 104 (50.2%) were females (table 3.1). The age of the subjects ranged between 1-83 years old with mean age of 21± 16 years old. The age groups were divided into 0-15, 16-30, 31-45, 46-60, 61-75 and 76-90 years old. The frequency of each group as follow: 92(44.4%), 68 (32.9%), 29(14.0%), 13(6.3%), 2(1.0%) and 3(1.4%) respectively (table 3.2).

Table (3.1): Frequency of gender

Gender	Frequency	Percentage (%)
Male	103	49.8 %
Female	104	50.2 %
Total	207	100 %

Table (3.2): Frequency of age groups

Age groups (years)	Frequency	Percentage (%)
0-15	92	44.4%
16-30	68	32.9%
31-45	29	14.0%

46-60	13	6.3%
61-75	2	1.0%
76-90	3	1.4%

3.2 Overall prevalence of *Plasmodium* species:

Out of 207 subjects, 175 (84%) were positive for *P.falciparum*, 13 (6.3%) were positive for *P.vivax*, 17 (8.2%) were positive for mixed infection of *P.falciparum* and *P.vivax*, 1(0.5%) was positive for mixed infection of *P.vivax* and *P.malariae* and 1(0.5%) was positive for mixed infection of *P.falciparum* and *P.malariae* (table 3.3).

Table (3.3): Prevalence of *Plasmodium* species

Species	Frequency	Percentage (%)
P.falciparum	175	84.5 %
P.vivax	13	6.3 %
P.falciparum and *P.vivax*	17	8.2 %
P.falciparum and *P.malariae*	1	0.5 %
P.vivax and *P.malariae*	1	0.5 %
Total	207	100.0 %

3.3 Prevalence of *Plasmodium* infections among age groups:

The results showed that the highest prevalence of *Plasmodium* species infection was in age group 0-15 years which comprised 44.4% of all studied population, followed by 32.9% in 16-30 age group, then 14.0 % in 31-45 more than thirty years. Using Chi-square test, the p-value was statistically insignificant (0.889). Table (3.4) showed the detailed prevalence of *Plasmodium* infections among the age groups.

Table (3.4): Prevalence of *Plasmodium* infections among age groups

Age groups	Species (%)					Total
	P.falciparum	*P.vivax*	*P.falciparum* and *P.vivax*	*P.falciparum* and *P.malariae*	*P.vivax* and *P.malariae*	
0-15	79 (38.2%)	3(1.4%)	9(4.3%)	1(0.5%)	0 (0.0%)	92 (44.4%)
16-30	54(26.1%)	6(2.9%)	7(3.4%)	0 (0.0%)	1(0.5%)	68 (32.9%)
31-45	24(11.6%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	29 (14.0%)
46-60	13 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (6.3%)
61-75	2 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
76-90	3 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.4%)

p=0.889

3.4 Hematological changes in each *Plasmodium* infection

An analysis of the hematological parameters was performed in each type of infection. The results showed that 45.9 % were anemic, due to *P.falciparum* infection. Thrombocytosis was detected in (46.4%) of *P.falciparum* infections (table 3.5). The results showed increased counts of TWBCs, lymphocyte, neutrophils and mixed cells (monocytes, eosinophils and basophils) in most of *Plasmodium* infections, but the highest counts were in *P.falciparum* infection which comprises the following: TWBCs (70.0%), lymphocytes (41.1%), neutrophils (66.2%) and mixed cells (62.3%). The difference in rates was statistically insignificant at p-value >0.05, as shown in (table 3.6).

Table (3.5): Hematological parameters of different types of *Plasmodium* infections

Parameters	Species (%)					P-value
	P.falciparum	*P.vivax*	*P.falciparum* and *P.vivax*	*P.falciparum* and *P.malariae*	*P.vivax* and *P.malariae*	
Hb g/dl:						
-Normal	80 (38.6%)	7 (3.4%)	7 (3.4%)	1 (0.5%)	0 (0.0%)	0.642
-Anemia	95 (45.9%)	6 (2.9%)	10 (4.8%)	0 (0 %)	1 (0.5%)	
Platelets:						
-Thrombocytopenia	51 (24.6%)	6(2.9%)	9(4.3%)	1(0.5%)	1(0.5%)	0.248

-Normal	28(13.5%)	1(0.5%)	3(1.4%)	0(0.0%)	0(0.0%)	
-Thrombocytosis	96(46.4%)	6(2.9%)	5(2.4%)	0(0.0%)	0(0.0%)	

Table (3.6): Hematological parameters of different types of *Plasmodium* infections

Parameters	Species (%)					P-value
	P.falciparum	*P.vivax*	*P.falciparum* and *P.vivax*	*P.falciparum* and *P.malariae*	*P.vivax* and *P.malariae*	
TWBCs:						
-Leucopenia	18(8.7%)	3(1.4%)	2(1.0%)	0(0.0%)	0(0.0%)	0.696
-Normal	12(5.8%)	2(1.0%)	0 (0.0%)	0(0.0%)	0(0.0%)	
-Leucocytosis	145(70.0%)	8(3.9%)	15(7.2%)	1(0.5%)	1(0.5%)	
Lymphocytes:						
-Lymphocytopenia	55(26.2%)	8(3.9%)	9(4.3%)	1(0.5%)	0(0.0%)	0.216
-Normal	35(16.9%)	2(1.0%)	3(1.4%)	0(0.0%)	0(0.0%)	
-Lymphocytosis	85(41.1%)	3(1.4%)	5(2.4%)	0(0.0%)	1(0.5%)	
Neutrophils:						
-Neutropenia	18(8.7%)	3(1.4%)	3(1.4%)	0(0.0%)	0(0.0%)	0.612
-Normal	20(9.7%)	10(4.8%)	14(6.8%)	1(0.5%)	1(0.5%)	
-Neutrophilia	137(66.2%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
Mixed:						0.956

-Decreased	19(9.2%)	3(1.4%)	2(1.0%)	0(0.0%)	0(0.0%)	
-Normal	27(13.0%)	2(1.0%)	3(1.4%)	0(0.0%)	0(0.0%)	
-Increased	129 (62.3%)	8 (3.9%)	12(5.8%)	1 (0.5%)	1 (0.5%)	

3.5 Relation between *Plasmodium* species and parasitemia among studied population

Low parasitemia (+) was reported in *P.falciparum* infection (43.5%). Mild parasitemia (++) was highest in *P.falciparum* infection (17.9%). Moderate parasitemia (+++) was 15.0% in *P.falciparum* infections followed by *P.falciparum* and *P.vivax* mixed infection (2.9%) and hyperparasitemia (++++) was mostly found in *P.falciparum* infection (8.2%). The difference in rates was found to be statistically highly significant at p-value=0.000 (table 3.7).

Table (3.7): Relation between *Plasmodium* species and parasitemia among studied population

Parasitemia	Species (%)				
	P.falciparum	*P.vivax*	*P.falciparum* and *P.vivax*	*P.falciparum* and *P.malariae*	*P.vivax* and *P.malariae*
+	90 (43.5%)*	7 (3.4%)	1 (0.5%)	0 (0.0%)	0 (0.0%)
++	37 (17.9%)	2 (1.0%)*	1 (0.5%)	0 (0.0%)	0 (0.0%)
+++	31 (15.0 %)	4 (2.0%)**	6 (2.9%)	0 (0.0%)	0 (0.0%)
++++	17 (8.2%)	0 (0.0%)	9 (4.3%)	1(0.5%)	1(0.5%)

p=0.000

*Include one case with gametocytes.

** Include two cases with gametocytes.

3.6 Relation between species, parasitemia and hemoglobin levels among studied population

There was no significant association between the types of malaria infections and hemoglobin levels, p-values of each *P.falciparum*, *P.vivax* and *P.falciparum* and *P.vivax* mixed infection were statistically insignificant (0.824, 0.721 and 0.652, respectively) (table 3.8). showed the relation between species, parasitemia and hemoglobin levels among study population**.**

Table (3.8): Relation between species, parasitemia and hemoglobin

levels(g/dl).

* Include one case with gametocytes.

** Include two cases with gametocytes.

3.7 Relation between species, parasitemia and total white blood cells among studied population

The results showed that most of *P.falciparum*, *P.vivax* and *P.falciparum* and *P.vivax* mixed infection had leucocytosis (82.9%, 61.5% and 88.2%, respectively). The difference in rates was statistically insignificant for the *P.falciparum* and

Parasitemia	Species (%)					
	P.falciparum n=175		*P.vivax* n=13		*P.falciparum* and *P.vivax* n=17	
	Hb levels (g/dl)		Hb levels (g/dl)		Hb levels (g/dl)	
	Normal	Anemia	Normal	Anemia	Normal	Anemia
+	41(23.4%)	49(28.0%)*	3(23.1%)	4(30.8%)	0 (0.0%)	1(5.9%)
++	16(9.1%)	21(12.0%)	1(7.7%)*	0 (0.0%)	0 (0.0%)	1(5.9%)
+++	17(16.8%)	15(8.6%)	1(7.7%)	2(15.4%)**	3(17.6%)	3(17.6%)
++++	7(4.0%)	10(5.7%)	0 (0.0%)	0 (0.0%)	4(23.5%)	5(29.4%)
Total	80(45.7%)	95(54.3%)	7(53.8%)	6(46.2%)	7(41.2%)	10(58.8%)
p-value	0.824		0.721		0.652	

P.vivax (0.324 and 0.783, respectively), while the mixed infection had a significant p-value (0.038) (table 3.9). showed the relation between species, parasitemia and TWBCs among studied population.

3.8 Relation between species, parasitemia and platelets count among population

The results showed that 54.9% had high platelets count due to *P.falciparum* infections, the p-value was statistically significant (0.000), while the other *Plasmodium* infections were statistically insignificant (p-values were 0.661 and 0.729 for *P.vivax* and *P.falciparum* and *P.vivax* mixed infection, respectively) (table 3.10) showed the relation between species, parasitemia and platelets count among studied population.

Parasitemia	Species (%)								
	P.falciparum n=175			*P.vivax* n=13			*P.falciparum* and *P.vivax* n=17		
	Leucopenia	Normal leucocyte count	Leucocytosis	Leucopenia	Normal leucocyte count	Leucocytosis	Leucopenia	Normal leucocyte count	Leucocytosis
+	5 (2.9%)	7 (4.0%)	78 (44.6%) *	2 (15.4%)	2 (15.4%)	3 (23.1%)	0 (0.0%)	0 (0.0%)	1 (5.9%)
++	5 (2.9%)	2 (1.1%)	30 (17.1%)	0 (0.0%)	0 (0.0%)	2* (15.4%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
+++	7 (4.0%)	1 (0.6%)	23 (13.1%)	1 (7.7%)	0 (0.0%)	3** (23.1%)	0 (0.0%)	0 (0.0%)	6 (35.3%)

++++	1 (0.6%)	2 (1.1%)	14 (8.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	8 (47.1%)
Total	18 (10.3%)	12 (6.9%)	145 (82.9%)	3 (23.1%)	2 (15.4%)	8 (61.5%)	2 (11.8%)	0 (0.0%)	15 (88.2%)
p-value	0.324			0.783			0.038		

Table (3.9): Relation between species, parasitemia and TWBCs among population

* Include one case with gametocytes.

** Include two cases with gametocytes.

Table (3.10): Relation between species, parasitemia and platelets count among population

Parasitemia	Species (%)								
	P.falciparum n=175			*P.vivax* n=13			*P.falciparum* and *P.vivax* n=17		
	Thrombocytopenia	Normal platelets count	Thrombocytosis	Thrombocytopenia	Normal platelets count	Thrombocytosis	Thrombocytopenia	Normal platelets count	Thrombocytosis
+	15 (8.6%)	11 (6.3%)*	64* (36.6%)	2 (15.4%)	1 (7.7%)*	4 (30.8%)	1 (7.7%)	0 (0.0%)	0 (0.0%)
++	5 (2.9%)	7 (4.0%)	25 (14.3%)	1 (7.7%)	0 (0.0%)*	1 (7.7%)	0 (0.0%)	0 (0.0%)	1 (5.9%)
+++	20 (11.4%)	5 (2.9%)	6 (3.4%)	3 (23.1%)	0 (0.0%)**	1 (7.7%)	3 (17.6%)	0 (5.9%)	2 (11.8%)
++++	11	5	1	0	0	0	5 (29.4%	2 (11.8%	2 (11.8%

	(6.3%)	(2.9%)	(0.6%)	(0.0%)	(0.0%)	(0.0%)	)	)	)
Total	51 (29.1%)	28 (16.0%)	96 (54.9%)	6 (46.2%)	1 (7.7%)	6 (46.2%)	9 (52.9%)	3 (17.6%)	5 (29.4%)
p-value	0.000			0.661			0.729		

* Include one case with gametocytes.

** Include two cases with gametocytes.

Chapter four

Discussion

This study was conducted among 207 subjects. Out of them 103 (49.8%) were males and 104 (50.2%) were females. This finding was similar to a previous study done in Nigeria by Erhabor *et al.* (2014) which showed that 53 % were males and 47 % were females out of 100 cases.

The study showed that 84.5% of the studied population were infected with *P.falciparum*, while 6.3% were infected with *P.vivax* and 8.2% had mixed infection with both *P.falciparum* and *P.vivax*. These results are comparable to the study carried out at Ahmedabad in by Shah *et al*. (2007) who showed that 80 % of cases were infected with *P.falciparum* and 18.7% of cases infected with *P.vivax* and no mixed infections were reported out of total 100 cases. The present study showed that there was only one case that has mixed infection of *P.falciparum* and *P.malariae* and another one case that has mixed infection of *P.vivax* and *P.malariae*. A previous study done by Ehrhardt *et al*. (2006) reported that the prevalence of *P.falciparum* and *P.malariae* mixed infection was 13.4 % out of 1015 patients.

The prevalence of *Plasmodium* infection in this study was highest among the 0-15 years old which was (44.4 %), followed by the second age group 16-20 years old which comprises 32.9% of *Plasmodium* infections, most of them were *P.falciparum* infections (38.2%, 26.1%, respectively). This study showed no relation between *Plasmodium* infections and age groups (p-value=0.889). These results were similar to the results of a previous study done in Nigeria which showed that 52.4% of children under 11 years were positive for malaria (Erharbor *et al*., 2014).

This study showed that *P.falciparum* cases had the highest percentage of low parasitemia (43.5%) followed by *P.vivax* (3.4%) while hyperparasitemia was high in *P.falciparum* cases (8.2%). The difference in rates was statistically significant at p-value =0.000. These results were similar to a previous study which showed that low parasitemia was high (53.4%) in *P.falciparum* cases, but was different when compared to parasitemia of *P.vivax* which was 76.3%. High parasitemia in *P.falciparum* was 15.3 %, without reporting of any gametocytes or mixed infection (Kotepui *et al*., 2015).

The present study showed that most of cases had high platelets count in different parasitemia levels of all *Plasmodium* infections. The p-value indicated strong association in case of *P.falciparum* infection (0.000). On the other hand, *P.vivax* and *P.falciparum-P.vivax* mixed infection have a higher p-values which indicates no association between parasitemia levels, platelets count and *Plasmodium* infections (p-values > 0.05). These results were different from another one which showed that there was a relation between platelets count and parasitemia of *P.falciparum* infection as well as *P.vivax* infection (p-value < 0.0001) (Kotepui *et al*., 2014).

The results showed that most of *Plasmodium* infections had high levels of leucocytes count in all levels of parasitemia. There was a statistically significant association between *P.falciparum* and *P.vivax* mixed infection and leucocytes count (p-value=0.038). Other infections had no statistically association (p-value>0.05). This result differed from a previous study done in India which reported leukopenia in 10.7% of 112 *P.falciparum* infected patients and in 15.2 % of 118 *P.vivax* infected patients. Their results showed that there was statistically significant differences in the leucocytes count in *P.falciparum* and *P.vivax* infections (p-value <0.0001) (Jadhav *et al*., 2003).

The results of this study showed that most of *Plasmodium* infections had low levels of hemoglobin and increased levels of platelets, leukocytes, lymphocytes, neutrophils and mixed cells count. There was no statistically significant association between *Plasmodium* infections and these parameters (p-value > 0.05). These results were comparable to a previous study done in Thailand which showed that there were insignificant associations between hemoglobin levels, leukocytes, differential counts and mixed infection of *P.falciparum* and *P.vivax* infections (p-value >0.05), while neutrophils and platelets count had a statistically significant association with these species (p-value < 0.05) (Kotepui *et al*., 2015).

Chapter five

Conclusion and recommendations

5.1 Conclusion:

The study concluded that *P.falciparum* infection cause significant hematological changes with platelets count in different levels of parasitemia. Also mixed infection cause significant hematological changes with total white blood cells count in different levels of parasitemia.

5.2 Recommendations:

- Further studies should be done on mixed infections to detect their effect on the complete blood count parameters.
- Further studies should be done on platelets functions test among malarial infected patients.
- Further studies should be done on blood count indices among malarial infected patients.
- Further studies should be done using advanced serological and molecular techniques in detection of malaria parasites.

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بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

M.Sc. in Medical Laboratory Science (Parasitology and Medical Entomology)

Questionnaire

Patients' data:

-Name: ..... -Patient ID: ..... -Age: .....

-Gender: Male: ….. Female: .....

Laboratory results:

-*Plasmodium* species: ……..

-Stage(s): …….

-Parasitemia:……

-Selected Complete Blood Count Parameters:

Hb: …….g/dl.

TWBCs: ……. $x10^9$/l.

Lymphocytes: ……. $x10^9$/l.

Neutrophils: …… $x10^9$/l.

Mixed cells: ……. $x10^9$/l.

Platelets count: ……. $x10^9$/l.

-Date: ..../..../2016 -Signature: .....