Sudan University of Science and Technology (SUST)

College of Graduate Studies

Estimation of Serum Ferritin Level among Malaria Patients in Khartoum

قياس مستوى الفرتين لدى مرضى الملاريا في الخرطوم

A dissertation submitted in partial fulfillment for the requirements of the M.Sc. Degree in Medical Laboratory Science -Parasitology & Medical Entomology

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بسم الله الرحمن الرحيم

الآية:

(الله نور السموات والأرض مثل نوره كمشكاة فيها مصباح المصباح في زجاجة الزجاجة كأنها كوكب دري يوقد من شجرة مباركة زيتونة لا شرقية و لا غربية يكاد زيتها يضيء ولو لم تمسسه نار نور على نور يهدي الله لنوره من يشاء و يضرب الله الأمثال للناس والله بكل شيء عليم)

صدق الله العظيم

(سورة النــور:35)

DEDICATION

To my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up.

To my brothers, sisters, relatives, mentor and friends who shared their words of advice and encouragement to finish this study.

To everyone who embarrassed me with his smile, it was very supportive.

To everyone who dedicated part of his time to read this thesis, I hope it will be useful to you.

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Abstract

This study was conducted in Khartoum state- Omdurman (Ombada hospital and Altohamy health center), during the period between August 2018 to April 2019.

The study is case- control study, one hundred and fifty individual were enrolled in this study, (100 malaria patients and 50 individuals as controls). 5ml of blood sample was collected from each participant and investigated for malaria infection by both microscopic examination and immunochromatographic test (ICT), serum ferritin level was estimated by (Mindray BS 200).

The results showed that 96% of malaria infection was due to *Plasmodium falciparum* and 4% was due to *Plasmodium vivax*.

The study showed that the mean of serum ferritin levels were decreased in malaria positive patients (45 ug/L in males, and 50 ug/L in females) when compared with healthy controls (87 ug/L).

The result demonstrated that the rate of malaria infection according to gender was almost close (51% males and 49% females).

The highest rate of malaria infection 30% was among the age group (21-30) years while the lowest rate 2% was at the age range of above 60 years.

Also the result showed that blood film showed a high detection rate compared with the ICT.

Conclusion:

The study conclude that, the serum ferritin levels decreased in malaria

patients.

المستخلص

أجريت هذه الدراسة في ولاية الخرطوم (مدينة أمدرمان), في مستشفى أمبدة النموذجي و مركز التهامي الصحي, في الفترة من أغسطس 2018 و حتى أبريل 2019 .

تضمنت الدراسة 150 شخص, (100 منهم مصابين بالملاريا, و 50 شخص أصحاء لعمل مقايسة), تم جمع 5 مل من الدم من كل الأشخاص الخاضعين للدراسة, و تم فحص الملاريا عن طريق الفحص المجهري لمسحات الدم المصبوغة و عن طريق الفحص السريع, و تم أيضاً قياس مستوى الفرتين.

النتائج أوضحت أن نسبة 96% من حالات الاصابة بطفيل الملاريا هي من نوع المتصورة المنجلية, بينما المتصورة النشيطة كان تمثل 4% من الحالات المصابة.

في هذه الدراسة لوحظ أن متوسط مستوى الفريتين في الدم تقل عند الأشخاص المصابين بالملاريا (45 للذكور و 50 للنساء) مقارنة بالأشخاص الأصحاء (87).

الدراسة أوضحت أيضاً أن نسبة الاصابة بالملاريا متقاربة للجنسين (51% للذكور و 49% بالنسبة للاناث), و أكثر فئة عمرية تعرضا للاصابة (30%) هي الفئة (21-30) عاماً, بينما أقل الفئات تعرضاً للاصابة (2%) هي الفئة ذات أكثر من 60 عاماً.

و توصلت الدراسة أيضاً الى أن فحص الملاريا عن طريق مسحات الدم المصبوغة في الشرائح الزجاجية أكثر دقة من استخدام طريقة الفحص السريع.

خلصت الدراسة الى أن مستوى الفرتين في الدم يقل عند الأشخاص المصابين بالملاريا مقارنة بالأشخاص الأصحاء.

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CHAPTER ONE

Introduction

Malaria is a mosquito-borne disease caused by *Plasmodium* parasites. which are transmitted to people through the bites of infected female *Anopheles* mosquitoes, called malaria vectors (WHO/2017).

Almost of the world population (3.5 billion people) live in areas at risk of malaria transmission in 106 countries and territories (WHO/2013).

people with malaria often experience fever, chills and flu-like illness. When left untreated they may develop severe complications and die. In 2013 an estimated 198 million cases of malaria had occurred worldwide and 500,000 people died. Most of them children in Africa (about 1,500 cases) (CDC/2015).

Hematological changes are some of the most common complications in malaria, and they play a major role in malaria pathology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocyte. When used in combination with other clinical and microscopy, these parameters could improve malaria diagnosis in sub-patent cases. However, a few previous studies revealed that there was no significant difference in hematological parameters between malaria positive and negative patients. On other hand, filtration in a normal ferritin level was observed in subjects with malaria parasitemia, there was a negative correlation between ferritin concentration and malaria density while the serum ferritin levels increased with increasing malaria antibodies. High concentrations of ferritin are found in the cytoplasm of reticuloendothelial system, the liver, spleen and bone marrow. High ferritin levels main indicate iron over load without appearance liver damage, as may be noted in the early stages of idiopathic hemochromatosis ferritin levels in serum have also be used to evaluate clinical conditions not related to iron storage, including inflammation, chronic liver disease and malignancy (Sharma *et al.*, 2014).

Rationale

Malaria is important and most widely distributed parasitic infection in the tropical developing countries.

Hematological changes are some of the most common complications in malaria and the changes in ferritin level may reversely be affected by parasite density, therefore, this study was carried out to assess the effect of malaria parasites on serum ferritin level in Khartoum state (Omdurman).

Objectives

General objective:

- To estimate the serum ferritin level among malaria patients in Khartoum state.

Specific objectives:

- To estimate the serum ferritin level among malaria positive patients and compare it with the control group.
- To diagnose malaria parasite using microscopic examination and rapid diagnostic test (ICT).
- To determine the malaria infection rate according to gender and age group.

Chapter two

2.Literature review

2.1 Historical background:

Malaria is a major global public health problem all over the world. There are four species of malaria parasite (*P.falciparum, P.vivax, P.ovale* and *P.malariae*). *P.falciparum* species is the most predominant, and it was affirmed as fatal. *P.falciparum* positive patient exhibit several divergent value in their blood cell parameter. Blood is the most easily accessible diagnostic tissue. Changes in hematological parameters are likely to be influenced by any disease condition which affects the hemopoetic physiology at any level. This is likely to happen with an endemic disease such as malaria that affects the host homeostasis at various fronts resulting in a myriad of clinical presentation (Sharma *et al*., 2014)

2.2 Epidemiology:

According to the latest World Health Organization estimates, released in November 2018, there were 219 million cases of malaria in 2017 and 435,000 deaths. The African region accounted for most global cases of malaria (70%).

For the second consecutive year, the annual report produced by WHO reveals a plateauing in numbers of people affected by malaria in 2017, there were an estimated 219 million cases of malaria, compared to 217 million the year before. But in the years prior, the number of people contracting malaria globally had been steadily falling, from 239 million in 2010 to 214 million in 2015.

The report highlights some positive progress. The number of countries nearing elimination continues to grow (46 in 2017 compared to 37 in 2010). (WHO/2018)

2.3 Biology:

The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. 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. 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.

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. 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 (CDC/2016)

2.4 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. The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs. 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).

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

2.4.1.1 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, 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 (Gitau and Eldred, 2005).

2.4.1.2 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).

2.5 The life cycle of *Plasmodium*:

The malaria parasites exhibit a complex life cycle involving an *Anopheles* mosquito and a vertebrate host (Figure 1) (CDC/2015).

When an infected female mosquito bites a human, the *Plasmodium* Sporozoites migrate to the liver and invade hepatocytes, where they replicate as hepatic schizonts until several thousand merozoites are produced and released in the blood stream. In *P.vivax* & *P.ovale*, but not in *P.falciparum*, some liver parasites remain instead quiescent (hypnozoites), resuming replication, and infection after several weeks or months. Upon erythrocyte invasion in the bloodstream, *Plasmodium* parasites undergo asexual replication forming mature schizonts whose rupture releases merozoites that invade new erythrocytes. Some blood stage parasites differentiate instead into male and female gametocytes

that, when ingested in the mosquito blood meal, are activated to produce gametes. Gamete fusion in the insect midgut produces a zygote which develops into a motile ookinete, traversing the gut wall, and transforming into an oocyst, where 1000s of sporozoites are produced. The life cycle is closed when sporozoites, migrated from the ruptured oocyst to the mosquito salivary glands, are injected in a new human host by the insect bite (Siciliano *et al.*, 2015)

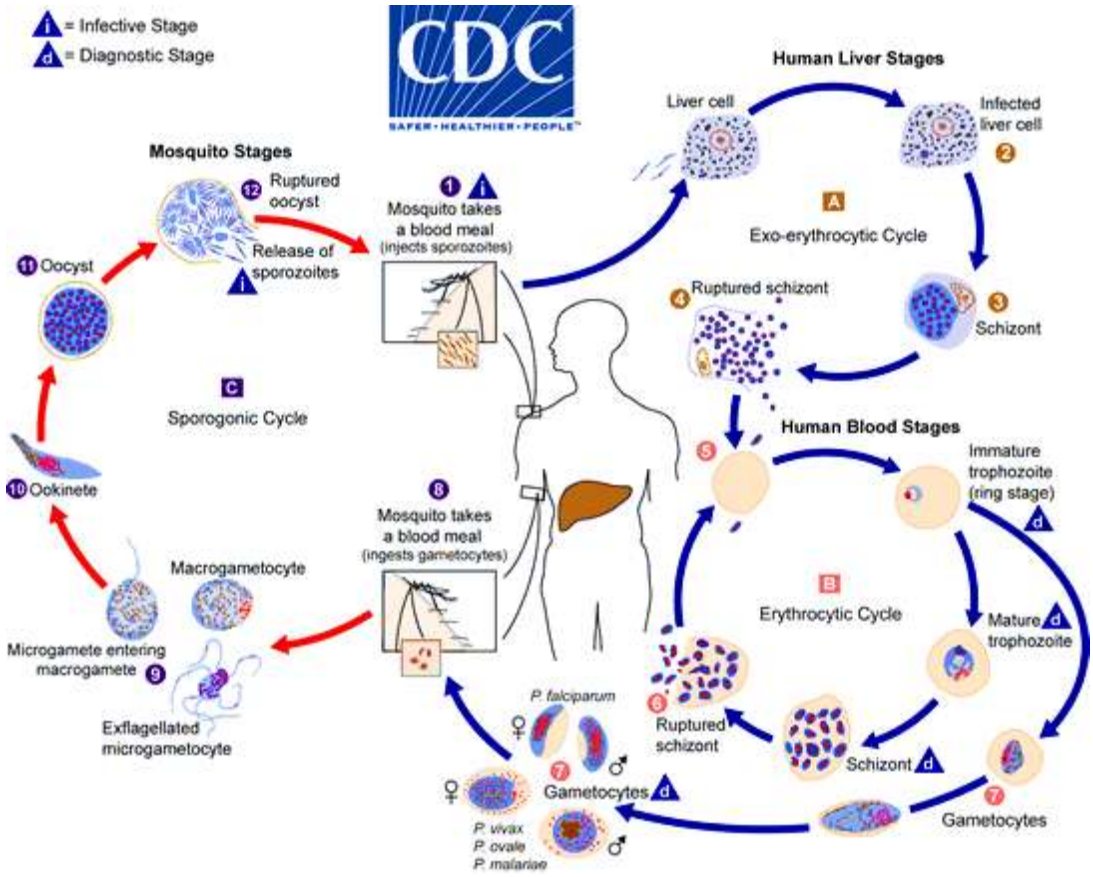


Figure (1): Malaria life cycle. (CDC/2015)

2.6 Pathogenesis and clinical features:

The disease process in malaria occurs due to local or systemic response of the host to parasite antigens. The typical presentation of malaria is periodic bouts of fever with chills and rigors. The febrile paroxysm follows the completion of erythrocytic schizogony when the mature schizont ruptures, releasing red cell fragments, merozoites, malaria pigments and other parasitic debris. It is commonly associated with

severe headache, nausea, and vomiting. Liver is enlarged and congested. Haemozoin pigments are found in the parenchymal cells. Spleen is soft, moderately enlarged, and congested in acute infection. In chronic infection, the spleen undergoes fibrosis and the sinusoids are dilated. Anaemia is caused by rupture of infected red blood cells and other causes of anaemia are by complement-mediated, autoimmune haemolysis and hypersplenism. A decreased erythropoiesis in the bone marrow may also contribute to anaemia (Mahmud *et al.*, 2017).

2.6.1 Pathogenetic characteristics of severe malaria:

2.6.1.1 Cytoadherence:

Cytoadherence, the ability of parasites to adhere to the vascular endothelium, was recognized as early as 1892 by Marchiafava and Bignami. Mature forms of parasites (asexual stage and gametocytes) can adhere to the vascular endothelium of several organs (lung, heart, brain, liver and kidney), the subcutaneous adipose tissues and the placenta. This feature of the disease in vivo has been related exclusively to *P.falciparum* However, sequestration in vitro to some endothelial cell lines and placental cryosections has also been seen in reticulocytes infected with *P.vivax* parasite sequestration is thought to be the pathological base of the severe manifestation of malaria, including cerebral malaria. It causes blood flow impairment leading to local hypoxia. It enhances parasite replication and the sticking of infected red blood cells (IRBC) to non-infected red blood cells. Moreover, when parasite sequester, the effects of parasite toxins are more localized and also the stimulation of the host immune response, which may cause a focused production of inflammatory mediators and tissue damage. As a consequence, both non infected RBC and IRBC become more rigid and less deformable. Sequestration is mostly mediated by mature parasite forms, approximately 20 hours after red blood cell invasion. The parasites produce new proteins that are exported to the IRBC surface and increase the adhesiveness of IRBC to the endothelium. During their 48-hour life cycle, the parasites can remain sequestered for 24 hours in the deep microvasculature. In this manner, they evade clearance by the spleen,

and make the diagnosis more difficult since they are not seen in the peripheral blood. Sequestration of *P.falciparum* has been attributed to different class of molecules of parasite origin and ligands present on the human endothelium. Among those, the *P.falciparum* histidine-rich protein (PfHRP) and the erythrocyte membrane protein 1 (PfEMP1), have received significant attention. PfHRP is related to the establishment of knobs, symmetric membrane arrangements which appear on the surface of infected RBC, while PfEMP1, a multimeric protein encoded by the var (variant) gene protrudes from the knobs and plays a major role in sequestration and thus virulence. To adhere to the endothelium, the parasites first adhere, roll and then become firmly attached to the endothelium adhesion molecules. Among these molecules, Intracellular adhesion molecule 1 (ICAM-1) a major sequestration receptor and involved in cerebral sequestration serves as a rolling receptor. On the other hand, cluster of differentiation 36 (CD36) gives stationary and stable adherence under flow. Sequestration is also seen during gestational malaria, when parasites adhere to the placenta. PfEMP1 is again the main adhesion receptor which adheres to the trophoblastic villous endothelium mainly through chondroitin-4-sulfate A (CSA) and other sugars such as glycosaminoglycans and possibly hyaluronic acid (HA).

Malaria in pregnancy can be severe for mothers and induce fetal death especially during the first pregnancy, when women usually lack sufficient immunity against CSA-binding parasites (Autino *et al.*, 2014).

2.6.1.2 Rosetting:

It refers binding of infected erythrocytes to uninfected erythrocytes. PfEMP1 also plays an important role in rosetting, as it can adhere to complement receptor 1 (CR1) and blood group A antigen present on the uninfected erythrocytes (Sastry *et al*., 2014)

2.6.1.3 Glycosyl phosphatidyl inositol (GPI):

Parasitic GPI stimulates the host immune system to release cytokines like interleukin 1 (IL-1), tumor necrosis factor (TNF) and interferon gamma (IFNg) (Sastry *et al*., 2014)**.**

2.6.2 Complications of falciparum malaria:

2.6.2.1 Blackwater fever:

This is a poorly understood condition, in which there is massive intravascular haemolysis and the passage of 'Coca-Cola'-coloured urine. Historically, this was linked to frequent quinine self-medication in expatriates living in malarious areas and indeed blackwater fever almost disappeared from Africa during the 'chloroquine' era from 1950 to 1980 but has since reappeared. Blackwater (urine) occurs in four circumstances when patients with G6PD deficiency take oxidant drugs (e.g. primaquine or sulphones), irrespective of whether they have malaria or not; occasionally when patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) deficiency have malaria and receive quinine treatment, and in some patients with severe *falciparum* malaria who have normal erythrocyte G6PD levels irrespective of the treatment given when people who are exposed to malaria selfmedicate frequently with quinine (or structurally related drugs). In severe malaria, rates of blackwater in Asian patients are similar whether the patients receive quinine or an artemisinin derivative. How quinine causes blackwater in these last three situations is not known, as it is not an oxidant drug. G6PD deficient red cells are particularly susceptible to oxidant stress as they are unable to synthesize adequate quantities of nicotinamide adinine dinucleotide phosphate (NADPH) through the pentose shunt. This leads to low intraerythrocytic levels of reduced glutathione and catalase and consequent alterations in the erythrocyte membrane and increased susceptibility to organic peroxides. Blackwater fever may be associated with acute renal failure, although in the majority of cases renal function remains normal (Farrar *et al.*, 2014).

2.6.2.2 liver dysfunction:

Jaundice is common in adults with severe malaria and there is other evidence of hepatic dysfunction, with reduced clotting factor synthesis, reduced metabolic clearance of the antimalarial drugs and a failure of gluconeogenesis which contributes to lactic acidosis and hypoglycaemia. Nevertheless, true liver failure (as in fulminant viral hepatitis) does not occur. There is sequestration in the hepatic microvasculature and, although many patients with acute falciparum malaria have elevated liver blood flow values, in very severe infections liver blood flow is reduced. In adults, liver blood flow values <15 ml/kg per minute are associated with elevated venous lactate concentrations, which suggests a flow

limitation to lactate clearance and thus a contribution of liver dysfunction to lactic acidosis. Direct measurements of hepatic venous lactate concentrations in severe malaria confirm that the hepatosplanchnic extraction ratio is inversely correlated with mixed venous plasma lactate. There is no relationship between liver blood flow and impairment of antimalarial drug clearance. Jaundice in malaria appears to have hemolytic, hepatic and cholestatic components. Cholestatic jaundice may persist well into the recovery period. There is no residual liver damage following malaria (Farrar *et al.*, 2014).

2.6.2.3 Hypoglycemia:

Hypoglycemia is associated with hyperlactatemia and shares the same pathophysiological etiology an increased peripheral requirement for glucose consequent upon anaerobic glycolysis (the Pasteur effect), the increased metabolic demands of the febrile illness, and the obligatory demands of the parasites which use glucose as their major fuel (all of which increase demand); and a failure of hepatic gluconeogenesis and glycogenolysis . Hepatic glycogen is exhausted rapidly: stores in fasting adults last approximately 2 days, but children only have enough for 12 hours. Healthy children have approximately three times higher rates of glucose turnover compared with adults, but in severe malaria turnover is increased by more than 50% (to values five times higher than those in adults with severe malaria). The net result of impaired gluconeogenesis, limited glycogen stores and greatly increased demand results in a hypoglycemia in 20–30% of children with severe malaria. In patients treated with quinine, this is compounded by quininestimulated pancreatic β-cell insulin secretion. Hyperinsulinemia is balanced by a reduced tissue sensitivity to insulin, which returns to normal as the patient improves. This probably explains why quinine-induced (hyperinsulinemic) hypoglycemia tends to occur after the first 24 hours of treatment, whereas malaria-related hypoglycemia is often present when the patient with severe malaria is first admitted. Hypoglycemia contributes to nervous system dysfunction and in cerebral malaria is associated with residual neurological deficit in survivors (Farrar *et al.*, 2014).

2.6.2.4 Metabolic (lactic) acidosis:

Metabolic acidosis is defined by an arterial blood pH of < 7.35 with a plasma bicarbonate concentration of < 22 mmol/L; and lactic acidosis is

characterized a pH < 7.25 and plasma lactate >5 mmol/L (Srinivas, 2015).

2.6.2.5 Hypotension and shock:

Systolic blood pressure being < 50 mmHg in children between 1-5 years, or <70 mmHg in patients ≥ 5 years; cold and clammy skin or core-skin temperature difference > 100C^{o} (Srinivas, 2015).

2.7 Laboratory diagnosis :

2.7.1 Microscopical examination:

Microscopy of peripheral blood thin and thick films remains the reference for malaria diagnosis. Although Giemsa staining is most commonly used, the Leishman staining method provides better visualization of the nuclear chromatin pattern of cells. It is less well known whether accuracy of parasitaemia assessment is equally accurate with the latter method (Sathpathi *et al*., 2014)

Detection of malaria parasites by light microscopy of Giemsa stained blood film remains the primary method for the diagnosis of malaria in health clinics and hospitals throughout the world. The quality of microscopy based diagnosis is frequently inadequate for ensuring good health outcomes. An acceptable microscopy service is one that is cost-effective, provides results that are consistently accurate and timely enough to have a direct impact on treatment. The effectiveness of malaria microscopy depends on maintaining a high level of staff competency and performance at all levels (Han *et al*., 2017)

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. The smear can be prepared from blood collected by vein puncture, finger prick and ear lobe stab (Srinivas, 2015).

2.7.2 Quantitative buffy coat test (QBC):

This method involved centrifuged and compressed red blood cell layer stained with acridine orange and then examined under an ultraviolet light source. The whole procedure takes place in a glass hematocrit tube which

is pre-coated internally with acridine orange stain and potassium oxalate; it is filled with 55-65µl of blood. The tube is centrifuged and so the components separate according to their densities forming bands. Fluorescing parasite are then observed, with UV microscope, at the red blood cell/white blood cell interface. QBC test is easier and faster than classic peripheral blood smear microscopy but the equipment required is expensive and species identification and accurate enumeration are impossible (Arora, 2010).

2.7.3 Rapid diagnostic tests (RDTs):

Rapid, simple and they do not need electricity. This tests are based on the detection of antigens or antibodies derived from malaria patients in lysed blood, using immuno-chromatographic methods. Most of frequently they employ a dipstick or test strip bearing monoclonal antibodies directed against the target parasite antigens. The tests can be performed in about 15. Several commercial test kits are currently available. The sensitivity of the RDTs has been most studied for *P.falciparum*, since the *P.falciparum* kits, targeting mostly *P.falciparum* HRP-2, have been available for a longer time. RDTs for *P.falciparum* generally achieve a sensitivity of >90% at parasite densities above 100 parasites per µl of blood. Below the level of 100 parasites per µl of blood, sensitivity decreases markedly. RDT sensitivity for non-*falciparum* species has been less extensively studied. The specificity of RDTs is also > 90%. The predictive value, both positive and negative, vary with parasite prevalence and are often found to be acceptable (Arora, 2010).

2.8 Treatment:

Malaria can be a severe, potentially fatal disease (especially when caused by *P.falciparum*) and treatment should be initiated as soon as possible. Patients who have severe *P.falciparum* malaria or who cannot take oral medications should be given the treatment by continuous intravenous infusion. Most drugs used in treatment are active against the parasite forms in the blood (the form that causes disease) and include:

- chloroquine.
- atovaquone-proguanil (Malarone).
- artemether-lumefantrine (Coartem).
- mefloquine (Lariam).
- quinine.
- quinidine.

- doxycycline (used in combination with quinine).
- clindamycin (used in combination with quinine).
- artesunate.

In addition, primaquine is active against the dormant parasite liver forms (hypnozoites) and prevents relapses. Primaquine should not be taken by pregnant women or by people who are deficient in G6PD (glucose-6-phosphate dehydrogenase). Patients should not take primaquine until a screening test has excluded G6PD deficiency (CDC, 2016).

2.9 Malaria control:

The main methods applied are Anopheles control by spraying houses and stables with insecticides (indoor spraying), environmental sanitation measures to eliminate mosquito-breeding places, and the usage of insecicide impregnated bed nets to reduce vector-human contacts. Further measures in endemic areas are early diagnosis and treatment of malaria cases as well as chemoprophylaxis in selected population groups. Antimalaria vaccines are not available yet. **(**Fritz *et al.,2005***)**

2.10 Malaria in Sudan:

Malaria in Sudan is a major public Health Problem. It leads to an estimated 7.5- 10 million cases and 35000 deaths every year. The burden of the disease on the health system is a reality. Out of the total outpatients' attendance, admissions and deaths malaria represents 20-40%, 30-50% and 15-20% respectively. These figures bring Sudan on the top of WHO / EMRO countries, as Sudan shouldered 50% of cases and 70% of deaths in the region (WHO, 2015).

2.11 Ferritin:

Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including algae, bacteria, higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, hence serum ferritin is used as a diagnostic test for iron-deficiency anemia (Wang *et al.,2010*)**.**

2.11.1 Protein structure:

Ferritins, highly symmetrical protein nanocages, are reactors for Fe2+ and dioxygen or hydrogen peroxide that are found in all kingdoms of life

and in many different cells of multicellular organisms. They synthesize iron concentrates required for cells to make cofactors of iron proteins (heme, FeS, mono and diiron). The caged ferritin biominerals, $Fe_2O_3 \cdot H_2O$ are also antioxidants, acting as sinks for iron and oxidants scavenged from damaged proteins; genetic regulation of ferritin biosynthesis is sensitive to both iron and oxidants. (Theil *et al.*, 2013)

2.11.2 Function:

2.11.2.1 Iron storage:

Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction. Hence vertebrates evolve an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored in a protein complex as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the reticuloendothelial system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the reticuloenothelial cells although hemosiderin is less readily available. Under steady state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin FR5Rl is the most convenient laboratory test to estimate iron stores. Because iron is an important mineral in mineralization, ferritin is employed in the shells of organisms such as molluscs to control the concentration and distribution of iron, thus sculpting shell morphology and colouration (Theil *et al*, 2013).

Iron is released from ferritin for use by ferritin degradation, which is performed mainly by lysosomes (Zhang, *et al*,2010).

2.11.2.2 Ferroxidase activity:

Vertebrate ferritin consists of two or three subunits which are named based on their molecular weight: L "light", H "heavy", and M "middle" subunits. The M subunit has only been reported in bullfrogs. In bacteria and archaea, ferritin consists of one subunit type.(Honarmand *et al*,2015). H and M subunits of eukaryotic ferritin and all subunits of bacterial and archaeal ferritin are H-type and have ferroxidase activity, which is the conversion of iron from the ferrous (Fe^{2+}) to ferric (Fe^{3+}) forms. This limits the deleterious reaction which occurs between ferrous iron and hydrogen peroxide known as the Fenton reaction which produces the highly damaging hydroxyl radical. ferroxidase activity occurs at a diiron

binding site in the middle of each H-type subunits. After oxidation of Fe(II), the Fe(III) product stays metastably in the ferroxidase center and is displaced by Fe(II), a mechanism that appears to be common among ferritins of all three kingdoms of life.(Honarmand *et al*.,2015) The light chain of ferritin has no ferroxidase activity but may be responsible for the electron transfer across the protein cage (Carmona *et al., 2014*).

2.11.2.3 Stress response:

The concentration of ferritin has been shown to increase in response to stresses such as anoxia; this implies that it is an acute phase protein (Honarmand *et al*.,2015).

2.11.2.4 Tissue distribution:

In vertebrates, ferritin is usually found within cells, although it is also present in smaller quantities in the plasma (Honarmand *et al*.,2015).

2.11.3 Diagnostic uses:

Serum ferritin levels are measured in medical laboratories as part of the iron studies workup for iron-deficiency anemia. The ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body. However, ferritin levels may be artificially high in cases of anemia of chronic disease where ferritin is elevated in its capacity as an inflammatory acute phase protein and not as a marker for iron overload (Theil, Elizabeth ,2012).

2.11.4 Normal ranges:

A normal ferritin blood level, referred to as the reference interval is determined by many testing laboratories. The ranges for ferritin can vary between laboratories but are usually between 30–300 ng/mL (=µg/L) for males, and 18–115 ng/mL (=µg/L) for females (Wang *et al*,2010).

2.12 Malaria and serum ferritin:

- Previous study in Ibadan, Nigeria showed that, serum ferritin levels decreased with increasing parasitaemia in the children (Chiaka *et al*.,2006).
- Other study was done in malaria patient in Assam, and found that , hematological abnormality and low serum ferritin level is observed as an imperative marker for identification of malaria patients (Sharma *et al*., 2014).

Chapter Three

3.Materials and methods

3.1 Study design:

This study was designed as a case control study.

3.2 Study area:

The study was conducted in Omdurman city (Khartoum state) in Ombada hospital and Altohamy health center, during the period between August 2018 and April 2019.

3-3 Study population:

This study conducted on patients with malaria and healthy individual free from malaria in Omdurman.

3-4 Sample size:

150 individual were enrolled in this study, (100 malaria patients and 50 healthy people as control).

3.5 Data collection:

Personal data obtained by standard questionnaire (appendix).

3.6 Data analysis:

The data were presented as frequencies, percentages and analyzed using Statistical Package for the Social Sciences program (SPSS program version 20), chi squire test.

3.7 Ethical consideration:

Approval of the ethical committee of the Sudan University of Science and Technology, College of medical laboratories was taken. Informed consent was obtained from all participants in the study.

3.8 Methods:

3.8.1 Collection of blood samples:

5 ml of venous blood were collected and placed in a blood plain container and EDTA anticoagulant, equal volume for each, 2.5 ml.

3.8.2 Preparation and staining of blood smears:

Three drops of collected blood were placed in clean and dry slide (about 2 cm from edge of slide) and then stirred by a corner of another clean and dry slide until appropriate thick smear obtained, the smear was left to dry. Drop of blood was placed on the middle of clean and dry slide and by edge of another slide placed just in front of the drop of blood and the spreader turned until it touched the drop of blood, then blood allowed to run along the edge of spreader, and then spreader was pushed forward to the end of the slide with suitable speed. The smear was left to dry. All thick and thin blood films were stained using Giemsa stain. Only thin films were fixed with methanol for 1-2 minutes The slides were covered with 10% Giemsa solution for 10 minutes. All slides were washed using clean water and allowed to air dry.

3.8.3 Examination of blood films:

3.8.4 RDTs (ICT for malaria):

Rite Sign Malaria Antigen *Plasmodium falciparum* / *Plasmodium vivax* test cassette contains a membrane strip which is pre coated with a monoclonal antibodies two separate lines. one monoclonal antibodies (test line p.f) are specific to the HRP-II (Histadine rich protein II) of *Plasmodium falciparum* ,and other monoclonal antibodies (test line p.v)are specific to the PLDH (plasmodium lactate dehydrogenase) of *Plasmodium vivax*.

3.8.4.1 Procedure:

Kit components and specimen were allowed to reach room temperature prior to testing. The test device were removed from foil, lace it on a flat,

dry surface. For whole blood specimen a pipette was used to transfer 5µl of whole blood to the specimen well, then add 4 drops of assay diluents vertically in square assay diluents well, after that waiting for a minimum of 15 minutes (up to 30) and we read the result**.**

3.8.4.2 Interpretation of result:

Positive: two or three distinct colored lines appear.

Mixed malaria infection: One line appears inthe control region, one line appear in *P.vivax* region and one line appears in *P.faciparum* line region.

P.falciparum infection: one line appears in the control region, and one line appears in *P.f*alciparum. region.

P.vivax infection: one line appears in the control region, and one line appears in *P.vivax* region.

Negative: only one colored line appears in the control region.

Invalid: control line fail to appear.

3.8.5 Serum ferritin:

3.8.5.1 Sample collection:

-Blood collected in plain container centrifuged at 4000 rpm for 5 minutes serum separated and stored at -20◦c.

-Hemolyzed or lipemic are excluded.

3.8.5.2 Diagnostic characteristics:

Ferritin is major iron storage compound in the body, its consists of protein shell enclosing a core of variable amount of iron. Ferritin is present at particularly high concentrations in lever, bone marrow and spleen.

Serum ferritin concentrations declines very early in the development of iron deficiency and it serves at a very sensitive indicator of iron deficiency. On the other hand, a large number of chronic infections (rheumatoid arthritis, renal disease), malignancies and patients with hemosidrosis, result in increased serum ferritin concentration.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

3.8.5.3 Reagent preparation:

Reagent prepared by pouring the contents of a reagent B (suspension of latex particles coated with anti-human ferritin antibodies. sodium azide 0.95 g/L) in reagent A bottle (Glycine buffer 170 mmol/L, sodium azide 0.95g/L , PH 8.2) and mixed thoroughly.

3.8.5.4 Examination:

The serum ferritin level measured by (Mindry BS-200).

Chapter Four

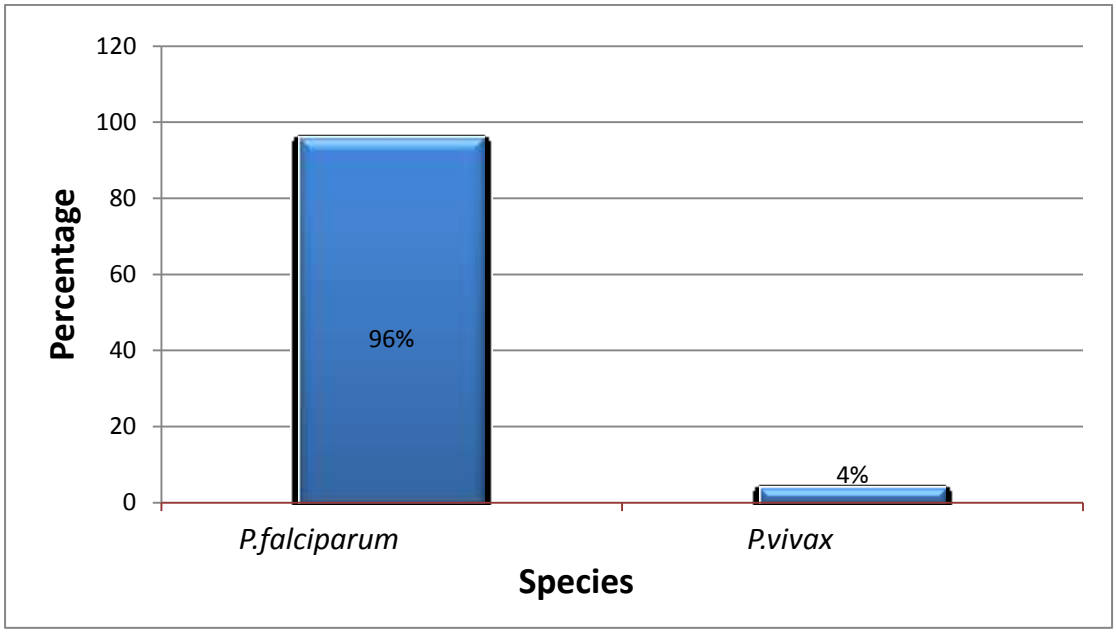
4.Results.

The results showed that out of the 100 patients infected with malaria, 96 were infected by *Plasmodium falciparum* (96%), and 4 were infected by *Plasmodium vivax* (4%) (table 1, figure 2).

Table (1): Frequency of *Plasmodium falciparum* & *plasmodium vivax* in the study groups.

Species

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Falciparum	96	96.0	96.0	96.0
	vivax	4	4.0	4.0	100.0
	Total	100	100.0	100.0	



Figure(2) : Frequency of *Plasmodium falciparum* & *plasmodium vivax*

Also the result showed that, the highest malaria infection rate (30%) was reported among the 21-30 years age groups, while the lowest rate (2%) was reported among the 61-70 years age group for malaria patients (table 2, figure 3).

Table (2) : The frequency of malaria infection according to age groups.

Age group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-10	18	17.5	18.0	18.0
	11-20	21	20.4	21.0	39.0
	21-30	30	29.1	30.0	69.0

31-40	14	13.6	14.0	83.0
41*50	7	6.8	7.0	90.0
51-60	8	7.8	8.0	98.0
61-70	2	1.9	2.0	100.0
Total	100	97.1	100.0	

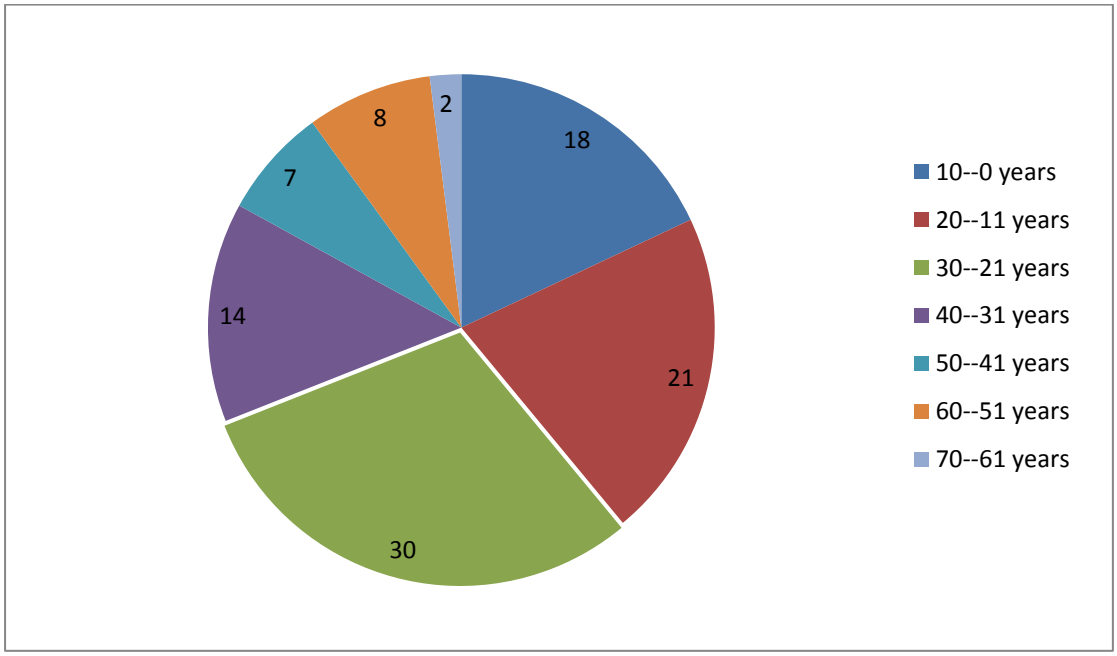


Figure (3) : Frequency of malaria infection in the study according to age groups.

Comparison between microscopic examination and ICT reveled that, out of 88 positive blood film, 45 were found to be negative by using ICT. On the other hand, 12 negative blood film were found to be positive by using ICT. This difference in value was statistically significant with p.value = .001 (table 3, figure 4).

Table (3): Comparison between BFFM and ICT results among malaria positive .

	ICT		
	Positive	Negative	Total

BFFM	Positive	43	45	88
	Negative	12	0	12
Total		55	45	100

p.value = .001

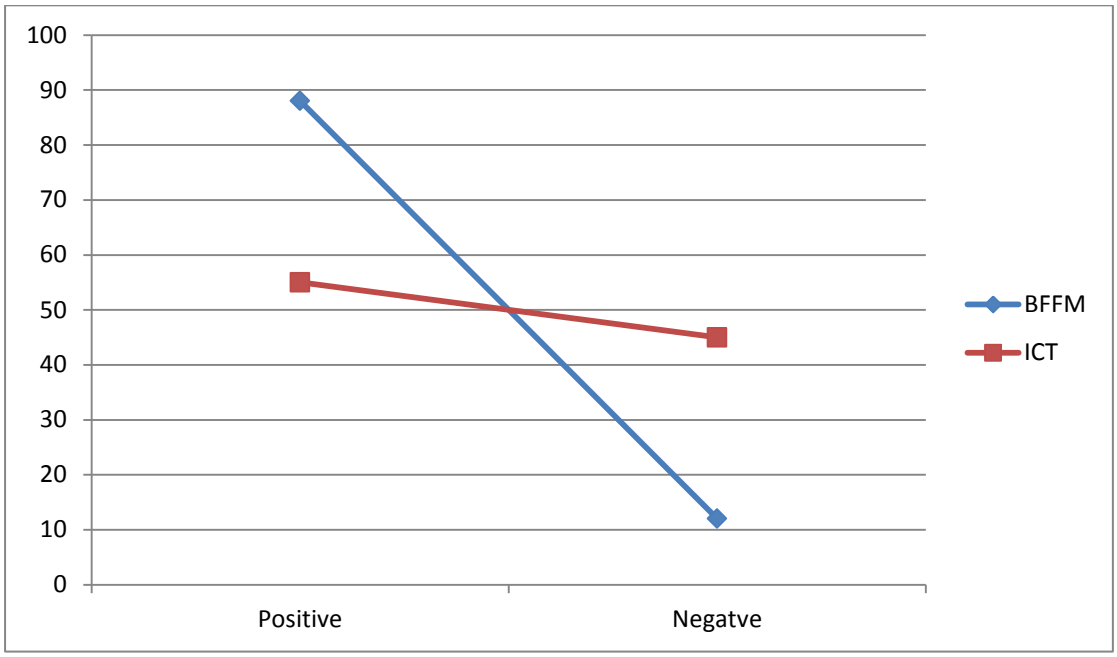


Figure (4) : Comparison between BFFM and ICT results among malaria positive.

In Malaria positive patients , The infection rates among males and females was almost close, 51% and 49% respectively (table 4)

Table (4): The Frequency of malaria infection in the study groups according to gender.

Gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	51	51.0	51.0	51.0
	female	49	49.0	49.0	100.0
	Total	100	100.0	100.0	

The mean serum ferritin levels of malaria positive males were 45.33 Ug⁄L in males and 49.88 Ug⁄L in females. The mean ferritin levels of control group was 87.23 Ug⁄L in males (n=31), and 87.42 Ug⁄L in females (n= 19)**.** (table 5&6).

The normal serum ferritin levels range from 12 to 300 Ug/L for males and 12 to 150 Ug/L for females.

Table (5): The results of serum ferritin in malaria positive group.

Gender	Mean	N	Std. Deviation
male	45.33	51	21.377
female	49.88	49	31.546
Total	47.56	100	26.805

Table (6): The results of serum ferritin in control group.

Gender	Mean	N	Std. Deviation
Male	87.23	31	54.593
Female	87.42	19	61.683
Total	87.30	50	56.767

Chapter Five
Discussion

In this study, the result revealed that, *Plasmodium falciparum* is the most dominant in the study area (96%), and *Plasmodium vivax* (4%), which was in agreement with (Han *et al*.,2017) "Comparison of microscopy and PCR for detection of human *Plasmodium* species and *Plasmodium*

knowlesi in southern Myanmar" which the percentage of infection is 67.8% *Plasmodium falciparum* and (30.5%) *Plasmodium vivax*.

In this study, it was seen that the, serum ferritin levels decreased in malaria positive patients when compared to healthy controls which agreed with the result of (Sharma *et al*,2014) "serum ferritin and hematological parameter among malaria patients in Assam".

Based on the results, the overall malaria infection according to gender was almost close (51% males and 49% 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.

This study evaluated the performance of different diagnostic tools in detecting malaria infection among patient.

The study showed the result of blood film as the gold standard was done when it was noted that it presented the high detection rate with rate (88 %). and ICT (45%) as low detection rate, p.value = .001. which agreed with the result of (Nado *et al*., 2004) which showed that microscopic examination (sensitivity 50%, specificity 100%) and ICT (37.5% sensitivity and 100% specificity).

The highest rate of malaria infection (96%) was among the age group (21-30) years, while the lowers rate (4%) at the age range of above of 60 years.

Conclusion

- Serum ferritin levels are decreased in malaria positive patients

- *Plasmodium falciparum* infection in the area investigated is more abundant than *Plasmodium vivax* infection.
- Age group of 21-30 years is the more susceptible to malaria infection than other age groups.
- Blood film showed a high detection rate compared with the ICT.

Recommendations

- Estimation of serum ferritin level is useful as supportive test for malaria diagnosis.
- The microscopic examination is the gold standard method for diagnosis of malaria.
- We recommend doing developing advanced researches about genetic and immunogenic composition of the malaria parasite and using of genetic techniques such as PCR and immunogenic techniques such as ELISA and IFAT in the malaria diagnosis.
- Control activities and advices should be conducted in the study area to reduce infection with malaria.

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Appendix I

Sudan University of Science and Technology
College of Graduate Studies

Questionnaire

No( )

Name:…….....

Age : .....

Gender:

Male ( ) female ( )

Diagnosis of malaria:

BFFM:

Positive ( ) Negative ( )

Species: .....

ICT for malaria:

Positive ( ) Negative ( )

Serum ferritin level: .....