بسم الله الرحمن الرحيم

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Evaluation of Auto Immune Haemolytic Anemia in Malaria Patients in Atbara City

A Thesis Submitted for the Partial Fulfilling of the MSc Degree in Haematology

By

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بسم الله الرحمن الرحيم

قال تعالى:

﴿ يَا أَيُّهَا النَّاسُ اتَّقُواْ رَبَّكُمُ الَّذِي خَلَقَكُم مِّن نَّفْسٍ وَاحِدَةٍ وَخَلَقَ مِنْهَا زَوْجَهَا وَبَثَّ مِنْهُمَا رِجَالاً كَثِيراً وَنِسَاء وَاتَّقُواْ اللّهَ الَّذِي تَسَاءلُونَ بِهِ وَالأَرْحَامَ إِنَّ اللّهَ كَانَ عَلَيْكُمْ رَقِيباً ﴾

صدق الله العظيم

سورة النساء الآية (١)

DEDECATION

TO

MY Mother

A strong and gentle who taught me to trust in Allah ,believe in hard work and that so much could be done with little

My Father

For earing an honest living for us and for supporting and encouraging me to believe in my self

My brothers and sister
Whom support and love me

AKNOWLEDGEMENT

Thanks to the **ALMIGHTY ALLAH** for giving me the strength and patience to complete this work successfully.

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for his unlimited support and guidance.

I would like to thank all those who assisted me, encouraged me to complete this research and provided me with the necessary information required to complete this study.

Abstract

Background:Autoimmune haemolytic anemia are caused by antibody production by the body against its own red cell they are characterized by a positive direct antigolobulin test, the aim of the study is to determine the possible association between auto immune hemolytic anemia and malaria.

Method : This is cross sectional study conducted in Atbara teaching hospital and selected health centers to determine the possible association between auto immune hemolytic anemia and malaria in the period between march 2018 to June 2018, the study include 60confromed as malaria malaria infected patents, blood samples were examined by immune-chormatographic test (ICT) for malaria, and direct anti globulin test (DAT), data was collected by using structured face to face questionnaires and analysis by SPSS version (11.5) program for statistical analysis.

Result: The study revealed that the malaria patients were 60% males and 40% females, the positivity among males 61.1% and females 39.9%, most common positivity at age (1 – 10 years) 38.9%.

The positivity among patients with plasmodium falciparum 61.1% are higher than patients cause of auto immune hemolytic anemia. Also patient with post infection with malaria (77.7%) is higher than other own don t infected with malaria before (22.2%), the anemic patient show (36.3%).

مستخلص:

فقر الدم المناعي الانحلالي الذاتي هو تكسر خلايا الدم الحمراء ذلك بسبب مهاجمة الجهاز المناعي الخاص بالجسم نفسه لهذه الخلايا بعض حالات المرض مجهولة السبب وبعض الحالات تسببها الأمراض والأدوية وتهدف هذه الدراسة الى تحديد احتماليه الارتباط بين فقرالدم المناعيالانحلاليالذاتي وعدوى الإصابة بالملاريا.

منهجيه الدراسة :اجريت هذه الدراسةالمقطعية في مستشفى عطبرةالتعليمي والمراكز الصحيةالمختارة لتحديد احتماليه فقر الدم المناعيالانحلاليالذاتي وعدوى الإصابة بالملاريا فيالفترة مابين شهر مارس ٢٠١٨ الي شهر يونيو ٢٠١٨وكانت عينه الدراسة عباره عن ٦٠ مريض تم اختيارها بصوره عشوائية وتم جمع عينات الدم من المرضى وتم تحليلها معمليا لإجراء فحص الملاريا السريع وفحص الكومس المباشر وتم جمع المعلومات بواسطه الاستبيان من ثم استخدام برنامج الحزمةالإحصائية للعلوم الاجتماعية الذى يعرف برنامج SPSS لتحليل بيانات الدراسة.

النتائج: أظهرت الدراسة أن المرضى ٦٠% منهم ذكور و٤٠% منهم اناث ان معدل الإصابة الموجب لفحص الكومس المباشرعند الذكور ٦١,١% وعند الاناث ٣٩,٩%والإصابة عند مرضى الفئه العمرية(١-١٠) سنه اكثر عرضه للإصابة ٣٨,٩% والإصابة بالطفيل المتصورةالمنجلية ٦١,١% والإصابة بالطفيل المتصورةالنشيطة ٣٨,٨% وان المرضى ذوى الإصابةالسابقة بالملاريا ٧٧,٧% مقارنه مع المرضى غير المصابين سابقا بالملاريا ٢٢,٢% ,مرضى فقر الدم ٣٦,٣%.

الخلاصة: مرض الملاريا مسئول بصوره جزئية عن فقر الدم المناعيالانحلاليالذاتي.

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List of Abbreviations

Abbreviations	Full name
AB	Antibodies
AIHA	Autoimmune hemolytic anemia
AMA	Apical membrane antigen
AMA – 1	Apical nerizoitic antigen – 1
ANA	Anti-nuclear antibodies
ANCA	Anti-neutrophil cytoplasm antibodies
APLA	Anit phospholipid antibodies
CA – AIHA	Cold antibodies Autoimmune hemolytic anemia
CSP	Circumsprozoite protein
DAT	Direct antiglobulin test
EBA	Erythrocyte binding site
ELISA	Enzyme likened immune sorbent assay
Hb	Hemoglobin
Hb s	hemoglobin S
HIF	Hypoxia inducible factor
HRP – 2	Histamine – rich protein
HSV – 1	Herpes simplex virus – 1
IG	Immunoglobulin
IGG	Immunoglobulin G
IGM	Immunoglobulin M
IL	Interleukin
IL – 10	Interleukin – 10
LSA	Liver stage antigen
MIF	Macrophage migration inhibitory factor
MSA	Merozoitic surface antigen

MSP	Merozoitic surface Protein
PCH	Paroxysmal cold hemoglobinemia
PCR	Polymerase chain reaction
RBC	Red Blood cell
RE	Reticulendothelial
RSV	Respiratory syncytial virus
SERA	Serine repeat antigen
TH_2	T helper $_2$ cell
TLR	Toll like receptor
TNF	Tumor necrosis factor
VZV	Varicella zoster virus
WA- AIHA	Wan antibody Autoimmune hemolytic anemia

Chapter One

Introduction

Justification

Objectives

1. Introduction, Rationale & Objectives

1.1Introduction:

Autoimmune haemolytic anemia is characterized by an increased breakdown of red blood cells (RBC) due to auto antibodies (auto-Ab's) with or without complement activation. The diagnostic features of AIHA include the combination of clinical and laboratory signs of RBC haemolysis together with the detection of auto-Ab's and/or complement deposition on RBC as mostly evidenced by a positive direct antiglobulin test (DAT) also known as direct Coombs test. A negative direct Coombs test using standard techniques does not exclude the diagnosis of AIHA **(**Packman CH.. 2008**)**

In more than 50% of the patients the development of AHIA is associated with an underlying disease *(secondary AIHA)*, but can occur without any evidence of an underlying disorder *(idiopathic or primary AIHA*)**(** Engelfriet CP, 1987) Based on the optimal temperature for autoantibody binding to RBC, AIHA is divided into a warm antibody AIHA (WA-AIHA), cold antibody AIHA (CA-AIHA) or AIHA due to biphasic auto-Ab (paroxysmal cold haemoglobinuria, PCH), With an incidence of 1:100,000 WA-AIHA is a rare disease, the incidence of CA-AIHA is even lower (1:1,000,000).**(**Packman CH. 2008**)**. In contrast, 10% of patients suffering from lupus erythematous develop an AIHA**(**Jeffries M, 2008 and Nossent JC1991**),**Occasionally, lymphoma is complicated by AIHA, but it can also be a herald of a lymphoma that has not yet been diagnosed. This is evidenced by the fact that 18% of patients with primary AIHA develop overt lymphoma at a later date**(** Arndt PA, 2009**).**

The pathology associated with exposure to *Plasmodium* differs significantly by species of *Plasmodium* parasite, age of the infected person, and intensity of transmission of the parasite. All infected hosts experience fever and chills associated with the periodicity of the rupture of the infected

erythrocytes. Differences in the symptoms and severity of disease caused by the different *Plasmodium* species**.**

Uncomplicated malaria occurs after infection with any *Plasmodium* species and is characterized by fever, chills and sweating in the majority of patients (Trampuz *et al*., 2003). If host immunity is adequate the infection can be cleared, but without treatment with effective antimalarial drugs, recrudescence of parasitaemia and relapse of symptoms can occur. Other symptoms include headache, nausea, malign, and vomiting (Trampuz *et al*., 2003). In contrast, complicated or severe malaria is characterized by anemia, cerebral malaria and metabolic acidosis, the main causes of death due to malaria infection. Other complications of severe malaria include pulmonary edema, acute renal failure, and hypoglycemia (Newton *et al*., 1998). The greatest burden of morbidity and mortality is associated with complicated falciparum malaria and the most vulnerable populations are pregnant women, children aged less than 5 years, and the elderly (Marsh *et al*, 1995). In sub-Saharan Africa where *P.falciparum* isholoendemic, more than 50% of children are parasitaemic at any given point in time (Høgh,1996) .Under these conditions, *Plasmodium* infection in children is recurrent, and the child's immune system is under constant stress from repeated *Plasmodium* infections (Marsh *et al*., 1995). Complicated malaria is associated with severe anemia, the major reason for malaria-related hospital admissions in Africa (Marsh *et al*., 1995). Severe malarial anemia is characterized by: haemoglobin, < 5 g/dL; haematocrit (erythrocyte volume fraction), < 15%; respiratory distress; and peripheral parasitemia (Marsh *et*18 *al*., 1995). The mechanisms of pathogenesis are poorly understood, but are linked to enhanced clearance of erythrocytes in the spleen from the periphery (Weatherall *et al*., 2002). Cerebral malaria is another complication of severe falciparum malaria and is linked to sequestration of the infected erythrocytes in the cerebral vasculature

(Weatherall *et al*., 2002),In sub-Saharan Africa where malaria transmission is endemic, pregnant women can be infected with *P. falciparum* and have evidence of infection of the placenta at the time of birth (Desai *et al*., 2007). Infection of pregnant women with *P. falciparum* has considerable adverse effects on maternal health and fetal morbidity and mortality (Desai *et al*., 2007; Rogerson *et al*., 2007), Infection of the placenta can cause intrauterine growth retardation and premature delivery, resulting in low-birth-weight infants or stillbirth (Desai *et al*., 2007), Although the greatest burden of placental malaria is in prim gravid women, women of all gravidities are at risk (Desai *et al*., 2007), While initial studies on placental malaria focused on birth outcomes, more recent studies have found that infants born to mothers with malaria have altered innate and adaptive immune responses (Ismaili *et al*., 2003). Several groups have reported that sensitization to parasitic antigens occurs *in uterus*, This exposure affects the development of specific antimalaria antibodies to prevent infection and also infant T-cell responses at birth, with a shift towards T-helper 2 (Th2) mediated responses in cord blood mononuclear cells from parasitized placenta (Dent *et al*., 2006; Metenou *et al*., 2007 and Flanagan *et al*., 2010),. Skewing of the gamma delta T-cell repertoire and altering responses to toll-like receptor (TLR) signaling in cord blood of infants born to mothers with placental malaria also indicate an effect of placental malaria on innate- immunity (Adegnika *et al*., 2008 and Cairo *et al*., 2008), Another mechanism by which placental malaria could affect infant immunity was suggested by the recent finding that tolerance to antigens encountered *in utero* develops through establishment of regulatory T cells (Mold *et al*., 2008), In support of this, cord blood of infants born to mothers with placental malaria showed an expansion of both malaria-specific and general regulatory CD4+ T cells, as characterized by expression of FOXP3 (Malhotra *et al*., 2009; Flanagan *et al*., 2010), Long-

term follow-up of these infants found that they had a greater risk of malaria over time, and evidence of T-cell anergy (Malhotra *et al*., 2009), Thus a consequence of exposure to malaria *in utero* could be significantly reduced ability of an infant to respond to *Plasmodium* and to other infections encountered early in life, and an increased susceptibility to infectious diseases encountered subsequently during infancy ,Placental malaria could have an additional role in increasing infant susceptibility to heterogonous infections. An important factor in protection against infections during infancy is the transfer of protective maternal antibodies across the placenta. However, several studies have demonstrated that placental malaria and maternal hyper-gammaglobulinaemia are associated with a significant reduction in the transfer of maternal IgG, including antibodies specific for measles, herpes simplex virus 1 (HSV-1), respiratory syncytial virus (RSV), tetanus, and varicella zoster virus (VZV) (Okoko *et al*., 2001a, b; Cumberland *et al*., 2007).

1.2 Rationale:

Malaria continues to be an important disease in human. Itsclinical manifestations are due to (a) invasion and destruction of RBCs by the parasite and (b) host reaction to the malarial parasite infection. Anemia, the most common complication, is due to accelerated RBC removal by the spleen, obligatory RBC destruction at parasite schizogony, and ineffective erythropoiesis. However the extent of haemolysis in malaria is much greater than encountered in other parasite induced hemolytic states. An associated or added immune mediated haemolysis has been postulated.

1.3 Objective:

1.3.1 General objective

- ✓ To determine autoimmune hemolytic anemia in malaria patients .

1.3.2 Specific objectives

- ✓ To Detect in vivo sensitization of RBCs with immune antibodies or the complement in malaria patient.
- ✓ To Examine the possibility that the type of parasite malaria rated to DAT.

Chapter Two

Literature Review

2. Literature review

2.1 Normal red cell destruction

Red cell destruction usually occurs after a mean lifespan of 120 days when the cells are remove dextravascularly by the macrophages of the reticuloendothelial(RE) system, especially in the marrowbut also in the nucleus, red cell metabolism gradually deterioratesas enzymes are degraded and not replaced and the cells become non-viable. The breakdown of heam from red cells liberates iron for recirculation via plasma transferr in to marrow erytlu'oblasts, and protoporphyrin which is broken down to bilirubin. Bilirubin circulates to the liver where it is conjugated to glucuronides which are excreted into the gut viabile and converted to stercobilinogen and stercobilin (excreted in faeces Stercobilinogen and stercobilin are partly reabsorbed and excreted, in urine as urobilinogen and urobilin. Globin chainsare broken down to amino acids which are reutilizedfor general protein synthesis in the body Haptoglobins are proteins present in normal plasma capable of binding haemoglobin. The haemoglobin haptoglobin complex is removed from plasma by the RE system. Intravascular haemolysis (breakdown of red cells within blood vessels) plays littleorno part in normal red cell destruction. (I.A.V.Hoffbrand, 2006)

2.1.1Introduction to haemolyticanemia's

Haemolytic anemia's are defined as those anemia that result from an increase in the rate of red cell destruction. Because of erytlu'opoietic hyperplasia and anatomical extension of bone marrow, red cell destruction may be increased several-fold before the patient becomes anemic-compensated haemolytic disease. The normal adult marrow, after full expansion, is able to produce red cells at 6-8 times the normal rate provided this is 'effective'. It leads toa marked reticulocytosis, particularly in the moreanemic cases. Therefore, haemolyticanemia maynot be seen until the

red cell lifespan is less than30 days**.(**I A.V. Hoffbrand,2006 **)**, There are two main mechanisms whereby red cells are destroyed in haemolyticanemia. There maybe excessive removal of red cells by cells of the R Esystem (extravascular haemolysis) or they maybe broken down directly in the circulation in a process known as intravascular haemolysis). Whichever mechanism dominates will depend on the pathology involved. In intravascular haemolysis, free haemoglobin is released which rapidly saturates plasma haptoglobins and the excess free haemoglobin is filtered by the glomerulus. If the rate of haemolysis saturates the renaltubular reabsorptive capacity, free haemoglobin enters urine and, as iron is released, the renal tubules become loaded with haemosiderin Methaemalbumin and haemopexin are also formed from the process of intravascular haemolysis.**, (**I A.V. Hoffbr and, 2006**)**

2.1.2 Autoimmune hemolytic anemia

Autoimmune hemolytic anemia (AIHA), caused by auto reactive red blood cell (RBC) antibodies along with clinical and laboratory evidence of hemolysis, is estimated to occur in approximately 1 in 80000 patientsannually.1 Although AIHA is rare, RBC autoantibody that bind to RBCs are frequently encountered in the blood bank laboratory. For example, 7.6% of all antibody workups at our institution involve RBC autoantibody, which is similar to the 7% incidence reported elsewhere.2 Although RBC autoantibody are seen in patients of all ages, a general increase in incidence is observed with age, most dramatically in patients older than 50 years.3 The RBC auto antibody are generally classified as either warm RBC autoantibody, if optimum reactivity with RBCs occurs at378C, or cold RBC auto antibodies, if optimum reactivity with RBCs occurs at less than 308C.The RBC auto antibodies can have varied clinical importance, ranging from insignificant to life-threatening AIHA .Because RBC auto antibodies impede RBC alloantibody identification and cross

matching of compatible units, timely blood-product distribution can be delayed, which becomes substantiated during urgent RBC transfusion situations ,such as bleeding and life-threatening anemia. As transfusion-medicine consultants, pathologists often have to integrate both laboratory and clinical findings and available resources to evaluate the risk of RBC transfusions and to recommend a course of action.

Autoimmune hemolytic anemia (AIHA), caused by auto reactive red blood cell (RBC) antibodies along with clinical and laboratory evidence of haemolysis, is estimated to occur in approximately 1 in 80 000 patients annually, (Petz LD, 2004.) Although AIHA is rare, RBC auto antibodies that bind to RBCs are frequently encountered in the blood bank laboratory. For example, 7.6% of all antibody workups at our institution involve RBC auto antibodies, which is similar to the 7% incidence reported elsewhere (Wheeler CA,2004). Although RBC auto antibodies are seen in patients of all ages, a general increase in incidence is observed with age, most dramatically in patients older than 50 years, (Blackall DP. 2007) The RBC auto antibodies are generally classified as either warm RBC auto antibodies, if optimum reactivity with RBCs occurs at378C, or cold RBC auto antibodies, if optimum reactivity with RBCs occurs at less than 308C.The RBC auto antibodies can have varied clinical importance ,ranging from insignificant to life-threatening AIHA ,Because RBC auto antibodies impede RBC allo antibody identification and cross matching of compatible units, timely blood-product distribution can be delayed, which becomes substantiated during urgent RBC transfusion situations ,such as bleeding and life-threatening anemia. As transfusion-medicine consultants, pathologists often have to integrate both laboratory and clinical findings and available resources to evaluate the risk of RBC transfusions and to recommend a course of action (Wheeler CA ,2004).

2.1.3 Clinical Feature

Clinically, AIHA and RBC auto antibodies present with signs and symptoms that reflect the activity and thermal reactivity of the autoantibody. For many patients, the presence of an RBC autoantibody alone may have no clinically apparent sequelae and will be detected only by an irregular antibody screen performed for another reason ,such as during a preoperative workup or during testing of blood after donation .Patients with AIHA associated with cold RBC auto antibodies have symptoms that reflect pathologic antibody characteristics. Cold RBC auto antibodies with broad thermal amplitude can fix complement when patients are exposed to cold temperatures, such as those occurring during winter months at northern latitudes, or when patients under go cooling during a cardiopulmonary bypass procedure. This phenomena most often occurs in a patient's extremities ,resulting in hemolysis and agglutination, and presents clinically as pallor, tachycardia, syncope, hypotension, and dyspnea (secondary to anemia), with red-brown urine and citrus (secondary to hemolysis), and cyanotic extremities(secondary to RBC sludging in the peripheral microvasculature).Patients with AIHA and warm RBC auto antibodies do not have the spontaneous agglutination of RBCs but can still have significant hemolysis as the RBCs coated with auto antibodies are removed by the reticuloendothelial system, which also presents with signs and symptoms of anemia and hemolysis ,In children, a special type of AIHA and autoantibody occurs, which clinically results in paroxysmal cold hemoglobinuria (PCH), a condition where a sudden presence of hemoglobin in the patient's urine is noted, often associated with a viral illness. This clinical presentation results from the causative biphasic immunoglobulin (Ig) G RBC autoantibody ,which is a unique cold-activating G-class immunoglobulin that binds to the P antigen on RBCs in low temperatures in the extremities and fixes complement, resulting in

hemolysis when RBCs are re warmed in the patient's core, Although nearly one-half of RBC auto antibodies arise in an idiopathic fashion, the remainder present in patients with underlying clinical conditions. For example, cold RBC auto antibodies may arise in patients with bacterial (Myco plasm a pneumonia) and viral (mononucleosis, Epstein-Barr virus) illnesses. Warm RBC auto antibodies have been found more frequently in patients with B-cell lymphomas (particularly chronic lymphocytic leukemia), macro globulinemias ,and autoimmune disorders like systemic lupus erythematosus .In a study categorizing 100 patients with warm auto antibodies, the most frequently associated clinical conditions were hematologic malignancies and autoimmune disorders followed by cardiac disease and non hematologic malignancies**.(** Wheeler CA, 2004**)**

2.1.4 Drug-induced immune haemolytic anemia's

Drugs may cause immune haemolytic anemia's via three mechanisms Antibody directed against a drug-red cell membrane complex (e.g. penicillin, ampicillin), Deposition of complement via a drug-protein (antigen)-antibody complex onto the red cell surface (e.g. quinidine, rifampicin), or 3 A true auto immune haemolytic anemia in which the role of the drug is lm clear (e.g. methyldopa).In each case, the haemolytic anemia gradually disappears when the drug is discontinued but with methyldopa the autoantibody may persist for several months. The penicillin-induced immune haemolytic anemia's only occur with massive doses of the antibiotic**, (**I A.V. Hoffbrand, 2006 **).**

2.1.5 Laboratary Finding

Screening for RBC auto antibodies is performed with the direct antiglobulin test (DAT). The DAT is often performed initially with a poly specific antihuman globulin (AHG) with reactivity for cells coated with sufficient immunoglobulin's or complement. If that test is positive with the poly specific reagent, then the patient's RBCs can be tested with

monospecific anti-IgG and anti-C3d to elicit the causative agent(s). The DAT reagent is incubated with the patient's RBCs, followed by examination for agglutination. In general, warm RBC auto antibodies result in a positive test when mono specific anti-IgG reagent is used, whereas cold RBC auto antibodies result in a positive test when mono specific anti-complement reagent is used because IgM is able to fix complement. The sensitivity of a positive DAT for RBC auto antibodies can depend on the reagents used as well as the technical expertise of the personnel performing the test**,(**Zantek ND,2012**)**Rare IgA RBC auto antibodies will not be detected by routine DAT testing ,True RBC auto antibodies have additional serologic characteristics. First, an eluate prepared from the patient's own RBCs should retract with the patient's RBCs, Plasma or serum from patients with a cold RBC autoantibody may have specificity for a carbohydrate antigen, such as i or I, and can react more or less strongly with group O panel cells that contain or lack such antigens, Warm RBC auto antibodies tend to have reactivity with common antigens on Rh proteins, and eluates prepared from patient cells containing a warm RBC autoantibody tend to be broadly reactive with all cells except the rare Rh-null cell ,Both warm and cold RBC auto antibodies can cause AIHA ,with the level of hemolysis reported to correlate with the strength of the DAT reaction, as well as reactivity with complement**,(**Blackall DP. 2007**)** Several other laboratory findings can be useful in determining the presence of hemolysis, For example, peripheral tests and complete blood cell counts typically reveal a normochromic, normocytic anemia ,polychromatic; and reticulocytosis. The RBC auto antibodies that result in a predominately extra vascular haemolysis can cause spherocytosis, predominately composed of micro spherocytes with a diameter of 5 lM or less ,Cold RBC auto antibodies can cause spontaneous agglutination ,observed as clumping of the RBCs on the test. Immune-mediated hemolysis can result in

hemoglobinemia, decreased serum haptogbin, increased serum total and un conjugated (indirect) bilirubin, as well as increased lactate dehydrogenase. Urinalysis may re veal hemoglobinuria and urobilinogen. Both clinicians and pathologists should understand that cold auto antibodies have the ability to interfere with hematology-based testing ,rendering the only reliable component in a complete blood cell count to be the hemoglobin .In the blood bank, during screening for irregular RBC antibodies, RBC auto antibodies can cause the auto control consisting of the patient's plasma and his or her own RBCs to agglutinate. Cold-reacting IgM RBC auto antibodies cause the auto control RBCs and the affected panel cells to agglutinate at room temperature and below when incubated with the patient's plasma or serum. The agglutination usually occurs without the addition of AHG because of therel actively large IgM pentamer, which cross-links with, and agglutinates, RBCs. In some cases, pre-warming the patient's specimen will abrogate the autoantibody-induced agglutination ,allowing the RBC allo antibodies to be identified so blood can be cross matched for transfusion. Aside from pre-warming the specimens, circumventing an offending cold antibody can be done by using 22% bovine serum albumin instead of low ionic-strength solutions because some cold RBC auto antibodies are enhanced by low ionic strength solutions**,** **(**Blaney KD, 2013**)**,Warm-reacting IgG RBC auto antibodies tend to cause all panel and auto control RBCs to agglutinate after incubation at 378C and the addition of AHG. Because all panel cells tend to agglutinate in the presence of warm RBC . auto antibodies and AHG, underlying RBC allo antibodies cannot be reliably detected. In addition, RBC units intended for transfusion are not cross match compatible in the presence of AHG because the warm RBC auto antibodies bind to antigens of the donor unit ,Several techniques are used to sequester the interfering autoantibody and thereby to look for the underlying allo antibodies. The optimal means of removing the

auto antibody is by adsorption. In this procedure, chloroquine or enzymes are used to remove bound autoantibody from the patient's RBCs, which are then incubated with a different sample of the patient's serum. This causes absorption of the autoantibody onto the patient's treated RBCs, leaving behind allo antibodies for further identification. If the patient has recently undergone transfusion, autologous adsorption cannot be performed, and instead, allogeneic RBCs with known antigen profiles can be used to absorb the autoantibody, allogeneic RBCs also adsorb any underlying allo antibodies if the RBCs contain the antigen recognized by the alloantibody. Therefore, only allo antibodies that react with antigens not on the adsorbing RBCs can be detected using this technique (Blaney, KD2013).

2.2 Malaria

Plasmodium is a genus of parasites belonging to the family Plasmodiidae, order Haemosporidia and phylum Apicomplexa. The genus Plasmodium is subdivided into 10 subgenera. (Cox-Singh et al., 2008and Kantele & Jokiranta, 2011), Malaria parasites in humans are all classified in the subgenera Plasmodium and Laverania. Four are well-characterized, strict human pathogens (e.g. P. falciparum, P. vivax, P. ovale, P. malariae), and one (P. knowlesi) is a recently identified human pathogen (Cox-Singh et al., 2008and Kantele & Jokiranta, 2011), Plasmodium has two obligate hosts in its life cycle: a mosquito host, which also serves as vector to a vertebrate host (Outlaw & Ricklefs, 2011), The insect vector of the parasite is used as one biological criterion for classification of the different species of Plasmodium (Outlaw &Ricklefs, 2011), Other biological criteria include the host range, the type of host cell infected, the length of the different stages of the life cycle, the presence or absence of relapse/recrudescence's, and geographical distribution (Outlaw &Ricklefs, 2011), The morphology of the parasite is also used to characterize species. Morphological criteria include the shape of the trophozoite, the gametocyte and the oocyst, the

number of nuclei in the erythrocytic and exo-erythrocyticschizonts, the aspect and distribution of the hemozoin pigment from the metabolism of the haemoglobin, and the nature of the damage induced by the parasite in the host cell (Outlaw &Ricklefs, 2011), As more Plasmodium species are sequenced, newer taxonomic criteria based on molecular characteristics such as the 18S small subunit rRNA, the genes for the circumsporozoite protein and for cytochrome b are now being included to define Plasmodium species and generate phylogenetic trees (Outlaw &Ricklefs, 2011), All Plasmodium species examined to date have 14 chromosomes, one mitochondrion and one plastid (Liu et al., 2010), Sequencing data have resolved the question of the origin of P. falciparum and its relationship with other Plasmodium parasites of primates and humans. Extensive sequence analysis of primate Plasmodium DNA indicates that P. falciparum is genetically related to a gorilla Plasmodium parasite in the Laverania subgenera (Liu et al., 2010).

2.2.1 Epidemiology and Transmission of *Plasmodium* infection

Plasmodium parasites are transmitted to humans by an infective bite from the Anopheles mosquito. The distribution of Anopheles species is highly variable from region to region, with considerable species variation between proximate geographical areas due to differences in environmental and climatic variables (e.g. altitude) that support or limit vector burden. Out of 390 species of Anopheles mosquitoes, only 50 species are known to transmit Plasmodium, with 20 species showing more localized global geographical distribution (Sinka et al., 2010a, b, 2011), The most common vectors of human Plasmodium parasites in many parts of Africa are Anopheles gambiae and Anopheles funestus (Sinka et al., 2010). Transmission intensity can be measured in numerous ways, including the entomological inoculation rate (EIR) (WHO, 2010). EIR is an estimate of the annual number of bites by infectious mosquitoes received by one

person (WHO, 2010). Alternative indicators of transmission intensity have been suggested, such as prevalence of antimalaria antibodies (Corran et al., 2007), but neither of these methods has been widely adopted in large-scale epidemiological studies (Gething et al, 2011), Holoendemic malaria transmission can be defined as parasite prevalence of > 70% and splenomegaly in > 80% of children. Shown in is a world map of P. falciparum endemicity (Gething et al., 2011), In this map, malaria endemicity has been categorized based on the age-standardized annual mean P. falciparum parasite rate in children aged 2–10 years (PfPR2–10), 10 spatial distribution of Plasmodium falciparum malaria in 2010 stratified by endemicity class the ability of the mosquito vector to survive in a given environment is a critical factor in determining the transmission intensity of malaria (Cooper et al., 2009), For example, malaria transmission has long been known to decrease with increasing altitude (Cooper et al., 2009), Transmission of P. falciparum is common at altitudes of < 1500 m (Cooper et al, 2009), However, episodic transmission of malaria has been reported in areas at altitudes above 2000 m, depending on vector dynamics and environmental influences (Cooper et al, 2009).

Modifiable factors that influence the existence of the disease include climate change, effective and available antimalarial therapy, intense use of chemically treated bed nets, and residual indoor spraying. Recent reports of reduction in the levels of P. falciparum transmission in Africa have been attributed to implementation of control strategies (WHO, 2016), but the risk of infection rebounds if vector-control strategies are relaxed.

2.2.2 Global distribution of Plasmodium infection in humans

Assessing the global epidemiology of malaria is a complex process involving evaluation of environmental ecology, vector-species identification, the local dominant parasites, dynamics of the exposed population, chemotherapeutic trends in clinical malaria, and the outcome of

control strategies (Ototo et al, 2011), Variation in the genetics of host susceptibility also contributes to the distribution of malaria globally (Ari et al, 2011). Overestimates of the burden of malaria can result when diagnosis is based on symptoms alone, since the symptoms typical of malaria (e.g. fever, chills, malaise) are also exhibited by other microbial infections characterized by acute febrile illness and the correct diagnosis of clinical malaria remains a challenge (Ari et al., 2011), In contrast, reliance on passive national reporting of malaria has likely led to an underestimate of the true burden of malaria worldwide (Snow et al., 2005), In 2015, 91 countries and areas had ongoing malaria transmission (WHO, 2017). Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places (WHO, 2017), Between 2010 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 21% globally (WHO, 2017), In that same period, malaria mortality rates among populations at risk fell by 29% globally among all age groups, and by 35% among children under five years (WHO, 2017). Sub-Saharan Africa carries a disproportionately high share of the global malaria burden (WHO, 2017). In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths (WHO, 2017),The Plasmodium species that infect humans are confined to the tropical and subtropical areas of the world where their insect vectors, mosquitoes of the genus Anopheles, are found. The large majority of infections can be linked to P. falciparum, with the remainder predominantly caused by P. vivax, and a very small number caused by P. malariae, P. ovale and P. knowlesi (WHO, 2011), The geographical distribution of Plasmodium species infecting humans.

2.2.3 Biological markers of infection and susceptibility

Diagnosis of the erythrocytic stage of infection can be a challenge in many countries. Routine laboratory diagnosis is done by microscopic examination to detect parasitized erythrocytes in Giemsa-stained thick and

thin films, from samples of blood obtained by finger prick (Krafts et al., 2011), however, this method relies on the availability of good microscopes, power sources, and trained microscopists. Because of these limitations, WHO has recommended that biomarker-based rapid diagnostic tests become the standard if feasible (WHO, 2010), These tests are based on presence of the parasite lactose dehydrogenase antigen or histidine-rich protein 2 (HRP-2) antigen in the blood (Wongsrichanalai et al., 2007). The sensitivity and specificity of these tests were found to be similar to those of microscopic detection (Abba et al., 2011). In the research setting, quantitative polymerase chain reaction (PCR) is used alongside microscopy (Malhotra et al., 2005), While serology is useful in understanding the epidemiology of malaria, it is not used diagnostically. Defining the correlations of protective humeral immunity to P. falciparum has presented a challenge to scientists for several reasons (reviewed in Marsh & Kinyanjui, 2006). This is even more evident in malaria holoendemic regions, where antibodies to P. falciparum are often short-lived in children (Kinyanjui et al., 2007; Crompton et al., 2010) and only increase with age and repeated infections (Crompton et al., 2010). Thus, because of the short half-life of immunoglobulin G (IgG)-specific antimalaria antibodies in children, use of antibodies as a marker of past exposure to P. falciparum is not always a reliable indicator. For example, in one study, children with a documented infection with P. falciparum and a P. falciparum IgG-antibody response to merozoite surface antigen 1 (MSP-119), (MSP-2) type A and B, apical merozoite antigen 1 (AMA-1) ectodomain, and region II of the 175 kDa erythrocyte-binding antigen (EBA-175II) was detected, but follow-up of the same children showed that the specific antibody response was lost (Kinyanjui et al., 2007), In addition, the choice of malaria antigen to be used as a marker of infection can be problematic because of the well-documented genetic variation in the surface antigens of P. falciparum

(Marsh &Kinyanjui, 2006). Several different assays have been used to measure antibody responses to P. falciparum (Marsh et al., 1989), but the most common is currently an enzyme-linked immune sorbent assay (ELISA), New technologies involving multiplex bead-based immunoassays have also been used and have the advantage of measuring more than one antigen in a limited sample volume (Asito et al., 2010), While no direct comparison was made in this particular study, other reports have found that sensitivity is similar to that of ELISA-based methods (Smits et al., 2012). Most epidemiological studies of P. falciparum infection and susceptibility are based on candidate vaccine antigens, because most research has focused on development of a vaccine against this parasite. Several different antibodies against antigens derived from either the blood stage [e.g. merozoite surface protein 1 (MSP-1), serine repeat antigen 5 (SERA5, SE36) and apical membrane antigen 1 (AMA-1)] or the liver stage of infection [e.g. liver stage antigen 1, LSA-1, circumsporozoite protein 1, CSP-1] have been evaluated, but the value of using these antibodies as markers of exposure to P. falciparum in children living in areas where holoendemic transmission occurs is unclear. However, measurement of antibodies to antigens derived from whole schizont extracts is thought to be a good estimate of past exposure to P. falciparum. The serology for detection of malaria infection is complicated, and no clear consensus has emerged about which antibodies are protective (Asito et al., 2010), The evolutionary burden of malaria on the human population is seen in the large number of inheritable genetic mutations occurring in erythrocytes including, for example, a variant of the haemoglobin beta gene, haemoglobin S (HbS) that causes sickle-cell disease in homozygotes. The advantage for the host is suggested by epidemiological studies demonstrating that heterozygotes for the sickle-cell gene (i.e. genotype HbAS, who exhibit the sickle-cell trait) are protected from the high parasite

densities and severe disease that characterize infection with P. falciparum (Lell et al., 1999 and Aidoo et al., 2002), Other human haemoglobinopathies such as thalassaemias and glucose 6-phosphate dehydrogenase deficiency also provide protection against infection. P. falciparum, the distribution of P. vivax in African populations may be 16 restricted by the absence of the erythrocytic Duffy antigen in many Africans. It has been suggested that the Duffy antigen is necessary for the entry of the P. vivax merozoite into an erythrocyte (Howes et al., 2011), although a very recent study has cast doubt on the strict requirement for this antigen for infection (Mendes et al., 2011).

2.2.4 Prophylaxis and treatment of malaria

The treatment of malaria targets primarily the blood stage of Plasmodium infection. While chloroquine has been the therapeutic drug of choice, increasing resistance among all Plasmodium spp., particularly P. falciparum, has led to the adoption of artemisinin-based therapy (Burki, 2011), However, resistance to artemisinins has now been reported in a growing number of countries in south-east Asia, and WHO recommends that oral artemisinin-based mono therapies be withdrawn from the market and replaced with artemisinin-based combination therapy (WHO, 2011). Intermittent preventive treatment of malaria has been recommended by WHO for preventive treatment of pregnant women and infants living in areas of high transmission of P. falciparum. Currently, 35 of 45 countries in sub-Saharan Africa and Papua New Guinea have adopted this policy. While intermittent preventive treatment is also recommended for infants living in regions of moderate to high malaria transmission, no countries have adopted this policy (WHO, 2011). The past decades have seen the introduction of insecticide-treated bednets (WHO, 2011), and it is estimated that the number of bednets in sub-Saharan Africa increased from 5.6 million in 2004 to 145 million in 2010, Studies on the efficacy of new

drugs continue because the number of drugs that treat malaria effectively is limited and because of resistance issues (Burrows et al., 2011), Primaquine is the only known drug that targets the liver stage of infection and eradicates hypnozoites ,however, primaquine can 21 cause haemolytic anemia in individuals with glucose-6-phosphate dehydrogenase deficiency, so widespread use of this drug for malaria-elimination campaigns is not feasible (Beutler & Duparc, 2007), There is no currently licensed vaccine for any of the human Plasmodium pathogens (Agnandji et al, 2011), A vaccine targeting the blood stage of P. falciparum was recently tested in phase III trials and found to be about 50% efficacious in preventing clinical and severe malaria in infants and children in Africa (Agnandji et al, 2011) .

Chapter Three

Materials and Methods

3. Material and Methods

3.1. Study design

This is a descriptive cross-sectional study to determine the possible associated between auto immune haemolytic anemia and malaria patients in Atabra city, during a period of(March 2018 ---- June 2018)

3.2. Study area

This study was conducted in Atabra City, is on the River Nile in Sudan, about 300 km from Khartoum.

3.3. Study population

This study was conducted on malaria infected patients.

3.4. Sample size

Sixty (n=60) malaria patients were recruited for this study.

3.5 Ethical consideration

Ethical approval to conduct this study in the region was obtained from the Health Services Director in Atbara locality and verbal consent was obtained from participants before collection of the blood samples.

3.6. Data collection

A structured questionnaire was used to collect demographic and clinical data.

3.7 Collection of blood specimens

Under sterile condition two ml of venous blood sample should be collected in EDTA container to prevent in vitro up take of complement

3.8 Laboratory investigation

3.8.1 Anti human globulin test

3.8.1.1 Principle

In 1945 Coombs, Mourant, and Race10 showed that red cells may combine with antibodies without producing agglutination. These investigators prepared an antibody that reacted with human globulins (e.g., a family of

human proteins) and used this reagent to agglutinate antibody-coated red cells. The reagent was called antihuman globulin (AHG),the procedure is referred to as the antiglobulin test. This test is applied to many blood banking testing protocols and provides important information. The antiglobulin test is important because it detects IgG antibodies and complement proteins that have attached to red cells either in-vitro or in vivo but do not show visible agglutination in testing .The principle of the antiglobulin test is not complicated. The test uses a reagent that has been prepared by injecting animals (e.g., rabbits) with human antibody molecules(human IgG) and complement proteins. In these animals, the injected proteins are recognized as foreign antigens, stimulating the animal's immune system to produce antibodies to human antibody molecules and complement proteins. The reagent, poly specific AHG ,contains antibodies to IgG molecules (anti-IgG) and complement proteins (anti-C3d, anti-C3b). This AHG reagent reacts with human IgG antibody and complement proteins whether freely present in serum or bound to antigens on the red cells. It is essential that red cells first be washed with physiologic saline to remove any unbound molecules before the addition of the AHG reagent. The washing step of an antiglobulin test requires the filling of test tubes with saline to mix with the red cells already present in the tube. These a line-suspended red cells are centrifuged. The saline wash is decanted from the red cell button, and this process is repeated for two to three additional cycles, On completion of the third or fourth wash, the saline is removed, and the tube is blotted dry to remove most traces of the saline, Red cell washing is an important technical aspect in the performance of an antiglobulin test. If the test red cells are inadequately washed, any unbound antibody or complement present in serum can potentially bind to the AHG reagent and inhibit its reaction with antibody or complement molecules attached to the red cells. This effect is known as

neutralization of the AHG reagent. Neutralization of the AHG reagent is a source of error in antiglobulin testing because it can mask a positive antiglobulin test.5 To detect potential neutralization, IgG-sensitized cells are added to tubes with negative reactions .After centrifugation, a positive reaction should be observed to confirm that washing was adequate ,After adequate red cell washing, the AHG reagent is added to the test. If the red cells in the test are sensitized with IgG or complement, the AHG reagent cross links and causes agglutination. The anti-IgG in the AHG reagent attaches to the Fc portion of the IgG molecule that is bound to the red cell, the anti-C3 in the AHG reagent attaches to C3molecules bound to the red cell as the consequence of complement activation. The formation of agglutinated red cells after the addition of AHG shows that IgG or complement proteins were attached to the red cells (Fig. 2-9). Agglutination is interpreted as a positive antiglobulin test. No agglutination at the completion of the antiglobulin test is interpreted as a negative antiglobulin test and indicates that no IgG or complement proteins were attached to the red cells ,Two types of antiglobulin tests are performed in the immune hematology laboratory:

Direct antiglobulin test (DAT) and indirect antiglobulin test (IAT). The distinction between these tests is often difficult for individuals entering this field because both tests use the AHG reagents. The DAT is a test in immune hematology to detect antibody bound to red cells in-vivo or ithin the body. In contrast, the IAT is used in immune hematology testing to detect antibody bound to red cells in-vitro or within a test tube.

3.8.1.2. Procedure

i. place 1 drop of 2 – 5 % suspension of red cell in clean labeled test tube .
ii. wash red cell 3 time with saline and decant the final wash completely .

iii. add 1 – 2 drop of anti human globulin reagent .

iv. mix and centrifuge 1000 rpm for 1 minute .

v. shake the tube gently to dislodge the button and read the result using a concave mirror .

vi. if result is negative , incubate the test for farther 5 min at room temperature then centrifuge and look for agglutination and record the result .

vii. add 1 drop of 5% of IgG sensitized red cell to the negative test look for agglutination if a negative result is obtain the test result is invalid and the test should be repeated .

viii. Appropriate controls are put with the test .

3.8. 2Immunochromatografic test – ICT - (Rapid test)

3.8.2.1 Principle

The malaria plasmodium falciparum and Plasmodium vivax rapid test cassette (whole blood) is a qualitative , membrane based immunoassay for detection of P. F and P.V antigen in whole blood . the membrane is pre-coated with anti-HRP-II antibodies and anti-PLDH during testing the whole blood specimen reacts with the dye conjugate which has been pre-coated on the test cassette. The mixture then migrates upward on the membrane by capillary action react with anti-histidine-Rich protein II (HRP-II) antibodies on the membrane of plasmodium falciparum test line region . If the specimen contain HRP-II or Plasmodium – specific Plasmodium vivax LDH or both , a colored line will appear in Plasmodium Falciparum line region or plasmodium vivax region or two colored line in plasmodium falciparum line region and plasmodium vivax line region . the absent of the color line in plasmodium falciparum line region or plasmodium vivax line region indicate that the specimen does not contain HRP-II and / or plasmodium – specific plasmodium vivax LDH . To serve as a procedure control a colored line will always appeared in the control line region

indicate that proper volume of specimen as been add and membrane wicking as occurred.

3.8.2.2 Procedure

i. Bring the pouch to room temperature before opening it . remove the test cassette from the sealed pouched and use it as soon as possible.
ii. Place the cassette on a clean and level surface for whole blood
iii. Using a pipette : to transferred 5 µL of whole blood to the specimen well , then add 3 drop of buffer (approximately 180µL)
iv. Used a disposal specimen dropper : hold the vertically , draw the specimen up the specimen well then add 3 drop of buffer and start the timer .
v. Wait for the colored line to appear. read result at 10 minutes do not interpret the result after 20 minute .

3.9 Data analysis

The data analysis was done through Statistical Package for the Social Sciences (SPSS) version 21 and Chi-square test was used to assess the association between various variables.

Chapter Four

Results

4. Results

Table (4-1) The Frequency and comparison of AIHA according to gender

Gender	Positive	Percent	Negative	Percent	Total
Male	11	61.1%	25	57.1%	36
Female	7	38.9%	17	42.9%	24
Total	18	100	42	100	60

P- Value = 0.889

Result indicate insignificant ,the positively among male 11 (61.1%) and among female is female 7(38.9%).

Table (4-2): The Frequency and comparison of AIHA according to age

Age groups	Positive	Percent	Negative	Percent	Total
1-10 year	7	38.9%	16	37.2%	23
11-20 year	3	16.7%	13	30.2%	16
21-30 year	3	16.7%	8	18.6%	11
31-40 year	0	0	1	2.3%	1
41-50 year	0	0	0	0	0
51-60 year	5	27.8%	4	11.6%	9
Total	18	100	42	100	60

P- Value = 0.485 result indicate in sig nificant ,the positively is most common at age, (1-----10) years is7 (38.9%) -he positively at age (51-60) years is 5(27.8%), and the positively at age (11-20) years and age (21-30) years is 3 (16.7%).

Table (4-3): The Frequency and comparison of AIHA according to Anemia

Result	Positive	Percent	Negative	Percent	Total
Yes	4	36.36%	14	28.57%	18
No	7	63.64%	35	71.43%	42
Total	11	100	49	100	60

P- Value = 0.018

Result indicate insignificant ,there is 4 patients anemic the positively shown (36.6%) ,14 patients no anemic but negative for AIHA (28.57%).

Table (4-4): The Frequency of AIHA according to drugs

Result	Positive	Percent	Negative	Percent	Total
Yes	0	0	18	30%	18
No	0	0	42	70%	42
Total	0	0	60	100	60

Table (4-5): The Frequency of AIHA according to Immunological disease

Result	Positive	Percent	Negative	Percent	Total
Yes	0	0	18	30%	18
No	0	0	42	70%	42
Total	0	0	60	100	60

Table (4-6): The Frequency and comparison of AIHA and Malaria Species

Result	Positive	Percent	Negative	Percent	Total
P.F	11	61.11%	30	71.43%	41
P.V	7	38.89%	12	28.57%	19
Total	18	100	42	100	60

P- Value = 0.346 result indicate insignificant ,the positively among patients with P. Falciparum are 11 (61.1%) and those infected with P.Vivax are 7 (38.89%)

Table (4-7): The Frequency and comparison of AIHA and post Malaria infection

Result	Positive	Percent	Negative	Percent	Total
Yes	14	77.78%	4	9.52%	18
No	4	22.22%	38	90.48%	42
Total	18	100	42	100	60

P- Value = 0.018result indicate in significant ,the positively patient alrealy infected with malaria is 14 (77.7 %) and patient with first infected with malaria is 4 (22.2%)

Chapter Five

Discussion
Conclusion
Recommendations

5. Discussion, conclusion and Recommendations

5.1Discussion

Auto immune haemolytic anemia is group of disease which have as their common factor the presence of auto antibobadies that bind to red cell and lead to premature reticuloendthelial system(KlempereMR.1984)

In this study the auto immune hemolytic anemia was assessed for 60 malaria patients at Atbara hospital and health centers ,only 18 (30%) were positive for AIHA .Result of the study agreed to study done by AHMerry,in Eastern Thailand the evidence of immune mediated haemolysis was sought in 73 patients with P.Falciprum malaria amongst 73patients with un complicated infection 12(16.4%) had weakly positivity DAT(AH.Merry-1986).

The incidence of AIHA according to age group is most highest in Age from (1 - 10 years) 7 (38 . 0 %) . from (11 – 20 years) 3 (16 . 7 %) . From (21 - 30 years) were 3 (16 . 7 %) . and from (51 - 60 years) were 5 (27 . 8 %). Result of the this current study are similar when compared to study done by CA.Facer, in West African(Gambian) that result obtain comparison of two groups of primary school children ,one from an area hyper endemic for malaria. and the other partially protected from malaria indicate an association between a high indicate of DAT positivity(CA.Facer. 1980)

The incidence of AIH in those whom infected which plasmodium falciparum is 11 (61.1%) higher than those whom infected with plasmodium vivax 7 (38 . 8 %) Result of the this current study are related when compared to study done be SAball, DJ Weeatherall in Gambian result ;the DAT was performed on 123 children with P.Falciprum malaria, 52 of children had a positive DAT (SAball.DJ -1982)

Also patient with post infection with malaria 14 (77 . 7 %) is higher than other whom don’t infected with malaria before 4 (22 . 2 %) .

The Anemic patient show 4 (36 . 3 %) positive For AIH ,because malaria infection in human by Plasmodium species is associated with reduction in hemoglobin level .

The incidence of AIHA among male 11(61.1%) and among female is 7 (38.9%)

5.2Conclusion

- In this study concluded that, the positively in male higher than female, and the children at the age (1—10 year)are most infected.

-P.Falciprum are most common cause of AIHA than P.Vivax.

5.3 Recommendations

1. People should be screened regularly for malaria infection.
2. For the studies should be conducted with larger sample size to confirm these results .
3. People show be recanted against malaria parasite and ensure they are assessed for Immunity (Post vaccination management).

Chapter Six

References

Appendix

6. References

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Appendix I

الاستبيان

الاسم.....

العمر .....

الجنس.....

هل أصبت بفقر الدم من قبل:

نعم ☐ لا ☐

هل أصبت بالملاريا من قبل:

نعم ☐ لا ☐

هل تعاني من احد الأمراض المناعية:

نعم ☐ لا ☐

هل تتعاطى أي نوع من الأدوية العلاجية:

نعم ☐ لا ☐

Appendix II

إقـــرار بالموافقـــة

الاسم :----------

العمر:---------- العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلــق بدراســة فقــر الــدم الانحلالي الذاتي لدى مرضى الملاريا في مستشفى عطبــرة التعليمــي والمراكــز الصحية المجاورة.

توحيدة حسين محمد الشيخ

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسديا ونفسي واعلم أن المشاركة فــي هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كمــا أنــه يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف:

د. حمزة أحمد حسن محمد التوم

التوقيع :---------- التاريخ:----------