بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Malaria Infection and Its Relation to ABO Blood Grouping in Giad and Al Gadeed Al Thawra Hospitals, Gezira State- Sudan

عدوى الملاريا وعلاقتها بفصائل الدم في مستشفيات جياد والجديد الثورة، ولاية الجزيرة- السودان

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

By

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قال تعالى:

(ربِ أوزعني أَن أشكُرَ نِعمَتَكَ التي أنعَمتَ عَليّ وَعَلى وَالِدَيَ وَأَن أَعمَلَ صالحاً تَرضَاهُ وَأَدخِلني بِرَحمَتِكَ في عِبادِكَ الصَالِحينَ).

صدق الله العظيم

سورة النمل الآية (١٩)

Dedication

To my lovely mother.

To my husband who encouraged me to complete this work.

To my colleagues and friends.

To my beautiful sons (Mohamed, Abu-Bakr, Omer)

To my sister and brother.

I dedicate this work.

Acknowledgement

First i thank Allah for granting me the strength to do this study.

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Abstract

This cross-sectional study was conducted in Giad and Al Gadeed Al Thawra hospitals in Gezira state to detect malaria infection and its relation to ABO blood grouping during the period from June 2017 to September 2018. The study was conducted on 280 subjects, 140 from each hospital. All samples were examined for malaria by using stained blood film and for blood grouping by using the slide method. The study showed that out of 280 samples examined, 100 (35.7%) were positive for malaria infection. The prevalence rates reported in Gaid and Al Gadeed Al Thawra were 36 (25.7%) and 64 (45.7%) respectively.

In Gaid hospital, from 140 subjects, 50 (35.7%) were males and 90 (64.2%) were females, the highest prevalence rate (30%) was reported among females while male reported (18%) prevalence rate. The highest prevalence rate (40%) was reported among the 16-30 years old and the lowest rate (16%) was reported among the 46-60 years old. The study revealed that the highest prevalence rate (31%) was reported among the A-ve blood group and the lowest rate (7%) was reported among the B-ve blood group. The results showed that the high parasitaemia (++++ and +++) was strictly confined to the A+ve and B+ve blood groups with rates of 17.1% and 12.5% respectively, while the low parasitaemia was more evident with the O+ve blood group with a 4.7% rate.

In Al Gadeed Al Thawra hospital, from 140 subjects, 60 (42.8%) were males and 80 (57.1%) were females, the highest prevalence rate (50%) was reported among males while female reported (42.5%) prevalence rate. The highest prevalence rate (71.1%) was reported among the 1-15 years old and the lowest rate (21.4%) was reported among the 46-60 years old. The study

revealed that the highest prevalence rate (57%) was reported among the O-ve blood group and the lowest rate (20%) was reported among the AB-ve blood group. The results showed that the high parasitaemia (++++ and +++) was strictly confined to the A+ve and B-ve blood groups with rates of 34% and 22.2% respectively, while the low parasitaemia (+) was more evident with the O+ve blood group with a 25% rate.

The study indicated that the prevalence of malaria infection in the study areas was high (35.7%). In addition, there was a significant relationship between the parasitaemia and blood grouping with p. value= 0.000.

مستخلص الدراسة

أجريت هذه الدراسة المستعرضة في مستشفيات جياد والجديد الثورة في ولاية الجزيرة للتعرف على عدوى الملاريا وعلاقتها بفصائل الدم في الفترة من يونيو ٢٠١٧ إلي سبتمبر ٢٠١٨م. أجريت الدراسة على ٢٨٠ شخص، ١٤٠ من كل مستشفى. كل العينات تمّ فحصها للملاريا باستخدام المسحة الدموية المصبوغة وفصائل الدم باستخدام طريقة الشريحة. أظهرت الدراسة أن من بين ٢٨٠ عينة تم فحصها، ١٠٠ (٣٥,٧%) كانت إيجابية لعدوى الملاريا. كان معدل الانتشار الذي سُجل في جياد و الجديد الثورة ٣٦ (٢٥,٧%) و٦٤ (٤٥,٧%) على التوالي.

في مستشفي جياد، من ١٤٠ شخص، ٥٠ (٣٥,٧%) كانوا ذكوراً و٩٠ (٦٤,٢ %) كانوا إناثاً، كان أعلى معدل انتشار (٣٠%) سُجل وسط الإناث بينما سجل الذكور (١٨%) معدل انتشار. كان أعلى معدل انتشار (٤٠%) سُجل وسط الفئة العمرية ١٦-٣٠ سنة و أدنى معدل (١٦%) سُجل وسط الفئة العمرية ٤٦-٦٠ سنة. أظهرت الدراسة أن أعلى معدل انتشار (٣١%) سُجل وسط الفصيلة (أ سالب) و كان أدنى معدل (٧%) سُجل وسط الفصيلة (ب سالب). أظهرت النتائج أن الإمراضية العالية (++++و +++) حُدِدت في الفصيلة (أ موجب) و (ب موجب) بمعدل ١٧,١% و ١٢,٥% على التوالي، بينما كانت الإمراضية المنخفضة (+) واضحة في الفصيلة (و موجب) بمعدل ٤,٧%.

في مستشفي الجديد الثورة، من ١٤٠ شخص، ٦٠ (٤٢,٨ %) كانوا ذكوراً و٨٠ (٥٧,١ %) كانوا إناثاً، كان أعلى معدل انتشار (٥٠%) سُجل وسط الذكور بينما سجلت الإناث (٤٢,٥%) معدل انتشار. كان أعلى معدل انتشار (٧١,١%) سُجل وسط الفئة العمرية ١-١٥ سنة و أدنى معدل (٢١,٤%) سُجل وسط الفئة العمرية ٤٦-٦٠ سنة. أظهرت الدراسة أن أعلى معدل انتشار

(٥٧%) سُجل وسط الفصيلة (و سالب) و كان أدنى معدل (٢٠%) سُجل وسط الفصيلة (أ–ب سالب). أظهرت النتائج أن الإمراضية العالية (++++و +++) حُدِدت في الفصيلة (أ موجب) و (ب سالب) بمعدل ٣٤% و ٢٢,٢ % على التوالي، بينما كانت الإمراضية المنخفضة (+) واضحة في الفصيلة (و موجب) بمعدل ٢٥%.

خلصت الدراسة إلى أن انتشار عدوى الملاريا في مناطق الدراسة كان عالياً بنسبة (٣٥,٧ %). بالإضافة إلى وجود علاقة بين الإمراضية و فصائل الدم بقيمة معنوية تساوي ٠,٠٠٠

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Chapter 1

Introduction

Malaria is a disease of global importance that result in 300-600 million cases annually and an estimated 2.2 billion people are at risk of infection (Singh *et al.*, 2010). Numerically the most important of the life threatening protozoan disease is malaria, which is responsible for at least 750,000 deaths a year, mostly in young children in Africa (Greenwood *et al.*, 2012; WHO, 2015). The agent of malaria is an obligate intracellular sporozoan in the genus *Plasmodium*, which contains four species: *P. malariae*, *P. vivax, P .falciparum* and *P. ovale* (Talaro and Chess, 2012). The human and some primates are the primary vertebrate hosts for these species, which are geographically separate and show variations in the pattern and severity of disease. Over half of the world's population is at risk from catching malaria. Malaria is currently endemic in 109 countries in four continents and of the 500 million cases of malaria estimated to occur annually, approximately one million results in death. Most of the fatalities are in children under the age of five years old and pregnant women (Lamb, 2012). Malaria accounts for at least $12 billion in economic losses each year in Africa and a reduction in annual economic growth estimated at 1.3 percent (NIH, 2010).

Blood is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. In vertebrates, it is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood (Igbenghu *et al*., 2012). ABO blood group system is genetically controlled and pro protein of various ABO groups differs significantly in different population and ethnic groups. Thus, any national or

international study reporting association of ABO groups with a disease must use population frequency of ABO groups as the base for comparison (Cserti and Dzik, 2007). Almost always, an individual has the same blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. Another more common cause in blood type change is a bone marrow transplant (Pathirana *et al*., 2005).

Rationale

Malaria in Sudan leads to serious health problems in community. A number of studies were conducted to detect the association between ABO blood grouping system and malaria. Despite the above researches, there is however, still lack of consensus on possible association between ABO blood group genes and malaria parasitaemia. This might be due to limited data on the association between malaria and red blood cell ABO antigens. So this study aimed at investigating malaria parasitaemia association with ABO blood groups among malaria patients in different endemic areas in Sudan.

Objectives

General objective:

To detect malaria infection and its relation to ABO blood grouping in Giad and Al Gadeed Al Thawra hospitals, Gezira state.

Specific objectives:

- To determine the prevalence rate of malaria infection in patients with different blood groups.
- To determine relationship between parasitaemia and different blood groups in patients with malaria.
- To determine malaria infection in study subjects according to age groups.
- To determine malaria infection in study subjects according to gender.

Chapter 2

Literature review

2.1 Malaria

2.1.1 Definition:

Earlier theories were that malaria was caused by bad air ("mala aria" in Italian) (CDC, 2012). The parasites in the blood were first seen in 1880 by French army surgeon Alphonse Laveran, who was looking for a bacterial cause of malaria. He is immediately realized that parasites rather than bacteria were responsible for the disease (Ridley, 2012). There are more than 100 species of *Plasmodium*, which can infect many animal species such as reptiles, birds and various mammals. Four species of *Plasmodium* have long been recognized to infect humans in nature. In addition, there is one species that naturally infects macaques which has recently been recognized to be a cause of zoonotic malaria in humans (CDC, 2012). The species infecting humans are: *P.falciparum*, which is found worldwide in tropical and subtropical areas. It is estimated that every year approximately 1 million people are killed by *P.falciparum*, especially in Africa where this species predominates. *P.vivax*, which is found mostly in Asia, Latin America and in some parts of Africa. Because of the population densities especially in Asia it is probably the most prevalent human malaria parasite. *P.ovale* is found mostly in Africa (especially West Africa) and the islands of the western Pacific. It is biologically and morphologically very similar to *P.vivax*. However, differently from *P.vivax*, it can infect individuals who are negative for the Duffy blood group, which is the case for many residents of sub-Saharan Africa. This explains the greater prevalence of *P.ovale* (rather than *P.vivax*) in most of Africa. *P.malariae*, found worldwide, is the only human malaria parasite species that has a quatrain cycle (three-day cycle). *P.knowlesi* is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques. It has recently been shown to be a significant cause of zoonotic malaria in that region, particularly in Malaysia. *P.knowlesi* has a 24-hour

replication cycle and so can rapidly progress from an uncomplicated to a severe infection; fatal cases have been reported (CDC, 2012).

2.1.2 Epidemiology:

Although malaria was once distributed throughout most of the world, the control of mosquitoes in temperate areas has successfully restricted it mostly to a belt extending around the equator. Despite this achievement, approximately 300 million to 500 million new cases are still reported each year, about 90% of them in Africa. The most frequent victims are children and young adults, of whom at least 2 million die annually. The case numbers in the United States, generally 1,000 to 2,000 new cases a year, are reported mainly in new immigrants (Talaro and Chess, 2012). The epidemiology of malaria is important in terms of understanding the basic immunological processes, as well as in deciding the control strategies to apply. It has been suggested that the clinical course of malaria is likely to differ according to the transmission in the area. Therefore, in areas where malaria transmission is seasonal and unstable, the disease burden is confined to a wide age range, and adults as well as children suffer severe morbidity. In areas with intense transmission, however, the burden of disease is confined to the youngest age groups, as adults would have already developed immunity and the highest incidence would be among children under 5 years old (Kayser *et al*., 2005).

2.1.3 Transmission:

Plasmodium parasites are transmitted to humans by female mosquitoes of the *Anopheles* genus. Worldwide, approximately 40 anopheline species have been documented to transmit parasites to humans. These species vary remarkably in their capacity to transmit *Plasmodium* to humans. This variability relates to basic habits, including tendency to seek out humans for a blood meal, types of water sources preferred for laying eggs, and predilection for entering human dwellings, as well as basic biologic capacity to support the development of human Plasmodia to development of mature infection that can be transmitted

during the taking of a blood meal (Hoffman *et al*., 2004). Anopheline species with the greatest competence to transmit *Plasmodium* are found in the Amazon (*Anopheles darlingi*) and Africa (the species complex of *An. gambiae*). Several identifiable factors characterize the capacity of *Anopheles* mosquitoes to transmit *Plasmodium*. The adult female mosquito can survive under ideal conditions up to 50 to 60 days. Practically, however, female anophelines survive on the average of 20 to 25 days, and consequently must be infected by malaria parasites in their early blood meals, taken every 3 to 4 days. Maturation of the parasites ingested with the blood meal requires 8 to 12 days before the mosquito has infective sporozoites in her salivary glands (Hoffman *et al*., 2004). Malaria can be transmitted by blood transfusion, needle stick injury, sharing of needles by infected drug addicts, or organ transplantation. The incubation period in these settings is often short because there is no pre-erythrocytic stage of development. The clinical features and management of these cases are the same as for naturally acquired infections (Hoffman *et al*., 2004).

2.1.4 Life cycle:

When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood (Srinivas, 2015). These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infested mosquito (Srinivas, 2015). When this female mosquito bites another man for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim, thus spreading the infection (Srinivas, 2015). The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood (CDC, 2016). In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites "merozoites" that continue the cycle

by invading other red cells. The blood stage parasites are those that cause the symptoms of malaria (CDC, 2016). When certain forms of blood stage parasites "gametocytes" are picked up by a female *Anopheles* mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito (CDC, 2016). After 10-18 days, the parasites are found as "sporozoites" in the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another human, the sporozoites are injected with the mosquito's saliva and start another human infection when they parasitize the liver cells (CDC, 2016). Thus, the mosquito carries the disease from one human to another (acting as a "vector"). Differently from the human host, the mosquito vector does not suffer from the presence of the parasites. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host (CDC, 2016). Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. Note that in *P.vivax* and *P.ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later. After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which

make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (figure 2.1) (CDC, 2016).

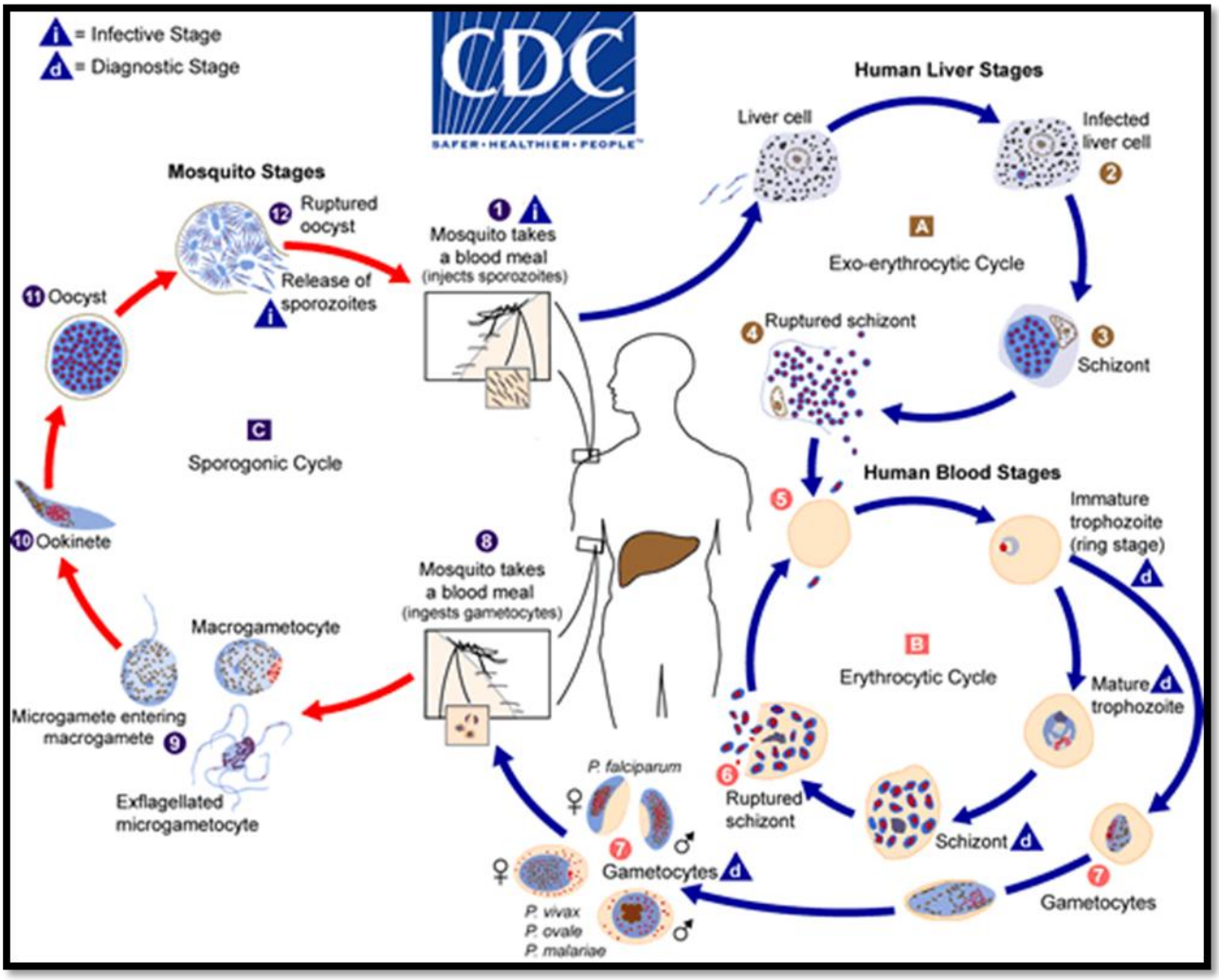

Figure (2.1): Life cycle of malaria (CDC, 2016)

2.1.5 Pathogenesis, pathology and symptomatology:

The pathogenic effect of a malarial infection have been considered to be directly related to hemolysis of infected red cell and uninfected cell, liberation of the metabolites of the parasite and the immunologic response of the host to this antigenic material and the formation of malarial pigment, additionally, in *falciparum* malaria the phenomenon of cytoadherence is basic to the locally diminished tissue perfusion seen in it is more severe complications, cytoadherence is the result of the expression on the surface of the parasitized red

cell of strain and stage-specific parasite-derived ligands, which adhere to a specific receptor complex on the endothelial cells. In persons subjected to repeat attack of malaria anaemia is disproportional to the number of red blood cells infected, and indicates that non infected red blood cells may become sensitized and be destroyed (John and Petri, 2006).

2.1.5.1 The classical symptoms of malaria:

The clinical symptoms of malaria are primarily due to schizont rupture and destruction of erythrocytes (Trampuz *et al.*, 2003). Malaria typically produces a string of recurrent attacks, or paroxysms, each of which has three stages-chills, followed by fever and then sweating. Along with chills, the person is likely to have headache, malaise, fatigue, muscular pains, occasional nausea, vomiting and diarrhea. Within an hour or two, the body temperature rises, and the skin feels hot and dry. Then, as the body temperature falls, a drenching sweat begins. The person, feeling tired and weak, is likely to fall asleep. The symptoms first appear some 10 to 16 days after the infectious mosquito bite and coincide with the bursting of infected red blood cells (RBCs). When many RBCs are infected and break at the same time, malaria attacks can recur at regular time periods-every two days for *P.vivax* malaria and *P.ovale* and every three days for *P.malariae* (NIH, 2009).

2.1.5.2 Severe malaria:

Almost all severe forms and deaths from malaria are caused by *P.falciparum*. Rarely, *P.vivax* or *P.ovale* produces serious complications, debilitating relapses and even death (Trampuz *et al.*, 2003).

2.1.5.2.1 Complications in patients with severe malaria:

2.1.5.2.1.1 Cerebral malaria:

This has a high case fatality and is a pathological condition resulting from infection with *P.falciparum*. A number of hypotheses have been proposed to explain the phenomenon of cerebral malaria, but in general it is thought to stem from immune responses against sequestered infected RBCs (Lamb, 2012). The

onset may be dramatic with a generalized convulsion, or gradual with initial drowsiness and confusion, followed by coma lasting from several hours to several days (Trampuz *et al.*, 2003). Sections of brain tissue from fatal *P.falciparum* infections reveal micro-vascular obstruction in the brain due to the accumulation of sequestered infected RBCs, autoagglutinates (where by infected RBC adhere to each other) and rosettes of infected RBCs, as well as infiltrates of lymphocytes. Brain-resident macrophages, or macrophage/ monocyte population that migrate to the brain tissue as a result of inflammatory immune responses against sequestered infected RBCs, directly contribute to the pathogenesis of cerebral malaria (Lamb, 2012).

2.1.5.2.1.2 Severe anaemia:

Severe malarial anaemia (SMA) is often associated with chronic and repeated infections of malaria, and it can lead to a drop in haemoglobin in the blood to <5 g/dl (normal value are between 10-15 g/dl for humans). Anaemia in malaria infection can be due to loss of RBCs during parasite replication, as well as removal of infected RBCs as part of immune -mediated clearance mechanism. In addition, increased phagocytic mechanisms in the spleen lead to premature clearance of un-infected RBCs; around ten times more uninfected RBCs are removed from the circulation that infected RBCs. RBCs loss is normally compensated for by the development and release of new RBCs from progenitor cells in a process known as erythropoiesis. Parasite products such as haemozoin, and anti-malarial immune responses to these products, can depress normal haematopoietic mechanisms in the bone marrow and spleen (Lamb, 2012).

2.1.5.2.1.3 Renal complications:

Acute renal failure is usually oliguric (<400 ml/day) or anuric (<50 ml/day), rarely nonoliguric, and may require temporary dialysis. Urine sediment is usually unremarkable. In severe cases, acute tubular necrosis may develop secondary to renal ischemia. The term 'blackwater fever' refers to passage of dark red, brown, or black urine secondary to massive intravascular hemolysis

and resulting hemoglobinuria. Usually, this condition is transient and not accompanied by renal failure (Trampuz *et al.*, 2003).

2.1.5.2.1.4 Pulmonary complications:

Acute lung injury usually occurs a few days into the disease course. It may develop rapidly, even after initial response to anti-malarial treatment and clearance of parasitaemia. The first indications of impending pulmonary edema include tachypnea and dyspnea, followed by hypoxemia and respiratory failure requiring intubation. Pulmonary edema is usually non-cardiogenic and may progress to acute respiratory distress syndrome (ARDS) with an increased pulmonary capillary permeability. Acute lung injury is defined as the acute onset of bilateral pulmonary infiltrates with an arterial oxygen tension/fractional inspired oxygen ratio of 300 mmHg or less, a pulmonary artery wedge pressure of 18 mmHg or less, and no evidence of left atrial hypertension. ARDS is defined as acute lung injury and an arterial oxygen tension/fractional inspired oxygen ratio of 200 mmHg or less. Volume overload and hypoalbuminemia may aggravate pulmonary capillary leakage. Chest radiograph abnormalities range from confluent nodules to basilar and/or diffuse bilateral pulmonary infiltrates. Non-cardiogenic pulmonary edema rarely occurs with *P.vivax* and *P.ovale* malaria (Trampuz *et al.*, 2003).

2.1.5.2.1.5 Metabolic acidosis:

The development of metabolic acidosis, whereby the pH of the blood lowers due to increased production of hydrogen in the body or defective removal of bicarbonate from the body by the kidneys, is often accompanied by respiratory distress and is strongly correlated with fatal malaria infection. Metabolic acidosis is exacerbated by the lack of circulating RBCs in patients with SMA, due to a reduction in the amount of oxygen delivered to the tissues and anaerobic metabolism. Hypovolaemia, whereby the volume of circulating blood decreases (presumably volume loss is partially due to lost RBC mass), is

associated with severe anaemia, and this also exacerbates metabolic acidosis (Lamb, 2012).

2.1.5.2.1.6 Hypoglycemia:

Hypoglycemia is a common feature in patients with severe malaria. It may be overlooked because all clinical features of hypoglycemia (anxiety, dyspnea, tachycardia, sweating, coma, abnormal posturing and generalized convulsions) are also typical of severe malaria itself. Hypoglycemia may be caused by quinine- or quinidine-induced hyperinsulinemia, but it may be found also in patients with normal insulin levels (Trampuz *et al.*, 2003).

2.1.6 Immunity to malaria:

Immunity produced following infection with malaria parasites is species-specific, stage-specific and strain-specific. Immunity in malaria is of two types:

2.1.6.1 Innate immunity:

This refers to inherent, non-immune mechanisms of host defense against malaria. This is due to: age of red blood cells, *P.falciparum* infects both young and old red blood cells while *P.vivax* and *P.ovale* infect only young erythrocyte and *P.malariae* only old erythrocyte. Nature of haemoglobin: presence of abnormal haemoglobin like thalassemia haemoglobin and foetal haemoglobin confers resistance against all plasmodium species, while sickle cell anaemia trait and haemoglobin E protect against *P.falciparum* and *P.vivax* respectively. Enzyme content of red blood cells: a genetic deficiency known as glucose-6-phosphate dehydrogenase (G6PD) trait confers some protection against *P.falciparum* infection. This enzyme is essential for respiratory process of the parasite. Presence or absence of certain factors: the presence of the Duffy factor increases the susceptibility to malaria. It is believed that Duffy factor present on the surface of erythrocytes acts as receptor for attachment of malaria parasite (Arora and Arora, 2010).

2.1.6.2 Acquired immunity:

Acquired immunity in malaria involves both humoral and cellular immunity. Antibodies against sporozoites and asexual and sexual blood stages develop in malaria patients. Antibodies (Immunoglobulin M (IgM), Immunoglobulin G (IgG) and Immunoglobulin A (IgA)) against asexual blood stages may protect by inhibiting red cell invasion and antibodies against sexual stages are believed to reduce malaria transmission. A variety of cellular mechanisms may play a role in conferring protection against malaria. These include natural killer activity and activated macrophages. The latter phagocytose and induce extracellular killing of target cells. T.cells are crucial for malaria immunity. Their major function seems to be providing help for the production of antibodies and to activate macrophages. Malaria parasites like many other micro-organisms are capable of periodically changing the expression of their antigens. This provides the parasite with a powerful means for evading host immunity. The ability of *P.falciparum* to remain sequestered by cytoadherence to the capillary lining of certain tissues is regarded as a selective advantage as such parasites can avoid frequent passage through spleen and thus exposure to immune effector mechanisms. Sequestration does not exist in other human malaria parasites and this is considered the main reason for the difference in disease severity (Arora and Arora, 2010).

2.1.7 Diagnosis:

Diagnosis of malaria involves identification of malaria parasite or its antigens in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors. The different forms of the four malaria species; the different stages of erythrocytic schizogony; the endemicity of different species; the population movements; the inter-relation between the levels of transmission, immunity, parasitaemia, and the symptoms; the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitaemia, and sequestration of the parasites in the deeper tissues; and the use

of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitaemia on a diagnostic test (Srinivas, 2015).The microscopic tests involve staining and direct visualization of the parasite under the microscope. For more than hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys (Srinivas, 2015). The useful examination of a well-prepared and well-stained blood film currently remains the "gold standard" for malaria diagnosis. The most commonly used microscopic tests include the peripheral smear study and the quantitative buffy coat (QBC) test (Srinivas, 2015). The simplest and surest test is the time-honoured peripheral smear study for malarial parasites. None of the other newer tests have surpassed the 'gold standard' peripheral smear study (Srinivas, 2015). Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. Thick smears are 20-40 times more sensitive than thin smears for screening of *Plasmodium* parasites, with a detection limit of 10-50 trophozoites/µl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitaemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes (Srinivas, 2015). The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitaemia. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear (Srinivas, 2015). The test takes about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentioned above. Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smear, which has a sensitivity of 90% (Srinivas, 2015). The level of parasitaemia 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 non-immune individuals, hyperparasitaemia (> 5% parasitaemia or > 250,000 parasites/μl) is generally associated with severe disease (Srinivas, 2015). The smear can be prepared from blood collected by vein puncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, ethmoid sinus via the nose or through fontanelle in young children can be used (Srinivas, 2015). Many of the new technologies for malaria diagnosis incorporate immunochoromatographic procedure, where conjugated monoclonal antibodies are the key reagents. Currently many rapid diagnostic tests (RDTs) are widely used for the diagnosis of malaria. These RDTs are simple lateral-flow immunochromatographic tests that detect parasite specific antigens released from red blood cells. Two of the tests, the ICT Malaria Pf/Pv and ParaSight-F detect histidine rich protein-2 (HRP-2), a protein produced by asexual stages and young gametocyte of *P.falciparum*. The third test OptiMAL detects *Plasmodium* lactate dehydrogenase (PLDH), a marker protein for the intra-erythrocytic form of the malaria parasite. HRP-2 is an abundant protein produced by all blood stages of *P.falciparum.* Also, there is insufficient data available to determine the ability of this test to detect the 2 less common species of malaria, *P.ovale* and *P.malariae* (Verma *et al*., 2013). Therefore, all negative RDTs must be followed by microscopy to confirm the result (CDC, 2014). Although the rapid diagnostic assays offer a number of attributes that make them attractive for use in the developing world (minimally trained personnel find them easy to use, no equipment is required, and samples can be read with the naked eye), they cannot quantify the level of parasitaemia or malarial species, they aren't reliable in the presence of low-level (and occasionally even

very-high-level) parasitaemia, they remain positive for 7 to 14 days after treatment (CDC, 2014). Alternative microscopic methods have been tried, including faster methods of preparation, dark-field microscopy, and stains like benzothiocarboxypurine, acridine orange and rhodamine-123. Acridine orange has been tried as a direct staining technique, with concentration methods such as thick blood film or the centrifugal quantitative buffy coat system and with excitation filter in the Kawamoto technique. Inability to easily differentiate the *Plasmodium* species, requirements of expensive equipment, supplies and special training as well as the high cost limit the use of these methods (Srinivas, 2015).

2.1.8 Treatment:

Malaria is an entirely preventable and treatable disease. The primary objective of treatment is to ensure a rapid and complete elimination of the *Plasmodium* parasite from the patient's blood in order to prevent progression of uncomplicated malaria to severe disease or death, and to chronic infection that leads to malaria-related anemia. From a public health perspective, treatment is meant to reduce transmission of the infection to others, by reducing the infectious reservoir and by preventing the emergence and spread of resistance to antimalarial medicines (WHO, 2016).

2.1.8.1Treatment of *P.falciparum* infections

World health organization recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by the *P.falciparum* parasite. By combining two active ingredients with different mechanisms of action, ACTs are the most effective antimalarial medicines available today (WHO, 2016). WHO currently recommends 5 ACTs for use against *P.falciparum* malaria. The choice of ACT should be based on the results of therapeutic efficacy studies against local strains of *P.falciparum* malaria (WHO, 2016). In low transmission areas, a single low dose of primaquine should be added to the antimalarial treatment in order to reduce transmission of the infection. Testing for glucose-6-phosphate dehydrogenase

(G6PD) deficiency is not required, as a single low dose of primaquine is both effective in blocking transmission at this low dose and unlikely to cause serious toxicity in individuals with any of the G6PD-deficiency variants (WHO, 2016).

2.1.8.2 Treatment of *P.vivax* infections:

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

2.1.8.3 Treatment of severe malaria:

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

2.1.9 Prevention and control:

Vector control is the main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. For individuals, personal protection against mosquito bites represents the first line of defense for malaria prevention. Two

forms of vector control are effective in a wide range of circumstances: insecticide-treated mosquito nets (ITNs): long-lasting insecticidal nets (LLINs) are the preferred form of ITNs for public health distribution programmes. WHO recommends coverage for all at-risk persons; and in most settings. The most cost effective way to achieve this is through provision of free LLINs, so that everyone sleeps under a LLIN every night. Indoor spraying with residual insecticides: indoor residual spraying (IRS) with insecticides is a powerful way to rapidly reduce malaria transmission. Its full potential is realized when at least 80% of houses in targeted areas are sprayed. Indoor spraying is effective for 3-6 months, depending on the insecticide used and the type of surface on which it is sprayed. Dicholoro diphenyl trichloroethane (DDT) can be effective for 9-12 months in some cases. Longer-lasting forms of existing IRS insecticides, as well as new classes of insecticides for use in IRS programmes, are under development. Anti-malarial medicines can also be used to prevent malaria (WHO, 2015).

2.2 Blood types:

A blood type (also called a blood group) is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or very closely linked genes) and collectively form a blood group system. Blood types are inherited and represent contributions from both parents. A total of 32 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT). There exist 4 major blood types; O, A, AB and B. These can be further subdivided into +/- (i.e., O+, A-), denoting the presence or absence of Rh antigen. Many pregnant women carry a fetus with a blood type different from their own, and the mother can form antibodies against

fetal RBCs. Sometimes these maternal antibodies are IgG, a small immunoglobulin, which can cross the placenta and cause hemolysis of fetal RBCs, which in turn can lead to hemolytic disease of the newborn called erythroblastosis fetalis, an illness of low fetal blood counts that ranges from mild to severe. Sometimes this is lethal for the fetus; in these cases it is called hydrops fetalis (Anstee, 2010).

2.2.1 The ABO blood groups system:

A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type is one of the many possible combinations of blood-group antigens. Across the 30 blood groups, over 600 different blood-group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups. Almost always, an individual has the same blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease (Anstee, 2010). Another more common cause in blood type change is a bone marrow transplant. Bone-marrow transplants are performed for many leukemias and lymphomas, among other diseases. If a person receives bone marrow from someone who is a different ABO type (e.g., a type A patient receives a type O bone marrow), the patient's blood type will eventually convert to the donor's type. Some blood types are associated with inheritance of other diseases; for example, the Kell antigen is sometimes associated with McLeod syndrome. Certain blood types may affect susceptibility to infections, an example being the resistance to specific malaria species seen in individuals lacking the Duffy antigen. The Duffy antigen, presumably as a result of natural selection, is less common in ethnic groups from areas with a high incidence of malaria (Anstee, 2010). If you have blood group A then you have got the A antigen on your red cells. Blood group B means you have the B antigen, while group O has neither, and group AB has both A and B antigens. The ABO system has associated anti-A and anti-B

antibodies, antibodies being the body's natural defense against foreign antigens. These antibodies are found in the plasma. Blood group A has the A antigen. This group recognizes the B antigen as foreign and can make anti-B antibodies. Similarly, blood group B has the B antigen and therefore recognizes the A antigen as foreign and can make anti-A antibodies. Group AB has both the A antigen and the B antigen so this group makes no antibodies. Group O has neither A nor B antigen so this group can be given safely to any other group. This is why Group O donors are known as "universal donors". Group O can make both anti-A and anti-B antibodies if exposed to these antigens. Giving someone blood from the wrong ABO group could be life-threatening. For instance, the anti-A antibodies in group B attack group A cells and vice versa. This is why group A blood must never be given to a group B person (Anstee, 2010).

2.2.2 Rhesus factor:

The Rhesus factor, also known as the Rh factor, is an antigen that exists on the surface of red blood cells in most people. People who have the Rhesus factor are considered to have a "positive" (+) blood type, such as A+ or B+. Those who don't are considered to have a "negative" (-) blood type, such as "O-" or "AB-." The Rhesus factor gets its name from experiments conducted in 1937 by scientists Karl Landsteiner and Alexander S. Weiner. Their experiments involved rabbits which, when injected with the Rhesus monkey's red blood cells, produced an antigen that is present in the red blood cells of many humans. Although there are at least 30 different systems for grouping blood types, most people are familiar with the ABO system, which groups blood into four general types: A, B, O and AB. Each blood type is usually further labeled as positive or negative, which is a reference to the Rhesus factor of the blood. More than 85% of people are Rh+ (Anstee, 2010).

2.2.2.1 The Rh factor and antigens:

The Rh blood grouping system actually involves more than 50 antigens that are found on the surface of red blood cells. These antigens are proteins that, when introduced into a body that does not have the same type, can cause the person's immune system to respond by producing antibodies that attack the proteins. The Rh factor, Rh+ and Rh-, usually refers specifically to the presence or absence of one of these proteins, the D antigen. The D antigen tends to cause an especially strong immune response in people who do not have it. There are two alleles, or genetic variants, of this antigen: D and d. A person who is Rh- has two recessive variants, dd. anyone who has at least one D= DD or Dd is Rh+. As with most genetic traits, one allele is inherited from each parent (Anstee, 2010).

2.2.3 Malaria evidence for selection of blood group phonotype that are rare outside area in which malaria is endemic:

It appears likely that the most devastating effects of malaria on human populations coincided with a change of lifestyle from hunter gatherer to more sedentary agricultural practices circa 10 000 years ago. The clearance of trees from forest areas created the potential for pools of stagnant water and breeding grounds for the mosquitoes carrying parasites. The clearest examples of selection in the face of malaria are reflected in the widespread distribution of inherited anemias, particularly sickle cell anemia and alpha thalassemia and the occurrence of hemoglobin C in regions of the world where malaria is endemic. The mutation giving rise to sickle cell disease (SCD; HbS) may have arisen at 3 different sites in Africa (Atlantic West Africa, Central West Africa, and Bantu-speaking Central and Southern Africa) with expansion of the mutation occurring 2000 to 2500 years ago. In this case, patients who inherit an HbS gene from both parents have SCD, whereas those who are heterozygous inheriting the HbS gene from 1 parent and the normal HbA gene from the other parent have substantial protection against malaria. A similar protective effect for the heterozygote seems likely in South East Asia, where HbE is very common and

red cells from patients of genotype HbAE are markedly less susceptible to malaria parasite invasion in vitro (Anstee, 2010). *P.vivax* and the blood group Fy (a-b-) phenotype complete absence from red cells of the molecule carrying the Duffy blood group antigens (aka DARC) is found in almost 100% of West Africans, and this absence is clearly and unambiguously demonstrated to provide protection from *P.vivax.* The molecular basis of this Duffy deficiency is a point mutation in the binding site for the transcription factor GATA-1. GATA-1 is a DNA-binding protein essential for erythropoiesis, and its failure to bind to the Duffy gene promoter means that the Duffy protein is absent from the red cells of affected subjects. In Africans the mutation occurs on a Duffy allele that would otherwise generate a Fy (b+) phenotype. The same GATA-1 mutation appears to have occurred on a second occasion in South East Asia, where it occurs on a Duffy allele that would otherwise generate a Fy (a+) phenotype. Another mutation creating weak expression of Duffy (Fy x) may also be relevant to malaria, but relevant population studies have not been reported. Recently, evidence for the emergence of *P.vivax* strains capable of invading Fy (a-b-) red cells has emerged in South America and East Africa (Anstee, 2010). The protective effect of the Fy (a-b-) phenotype against *P. vivax* is clear and unambiguously established. Not so clear are any deleterious consequences of this mutation for the subjects expressing the phenotype. Duffy protein is expressed on endothelial cells in these subjects but not on red cells, so any attempt to understand the consequences of red cell Duffy deficiency must take account of the functional role of endothelial Duffy. The Duffy protein is a member of the 7 membrane-spanning chemokine receptor families but unlike most chemokine receptors does not effect intracellular signaling through G proteins. It binds several pro-inflammatory chemokines of both the CXC and CC subfamilies but does not bind homeostatic chemokines. Recent evidence suggests Duffy protein on endothelial cells binds chemokines and facilitates leukocyte extra-vasation contributing to disease pathogenesis through

inflammation. Evidence for up-regulation of Duffy expression in the vascular endothelium during infection and transplant rejection supports this view (Anstee, 2010). The lack of Duffy on red cells in Fy (a-b-) patients alters the balance of pro-inflammatory chemokines in the body because the very large capacity of red cell binding is absent but the consequences of this change are presently unclear. Evidence that red cell and endothelial Duffy regulate the kinetics of chemokine bioavailability between the circulation and extravascular sites during inflammation. Clearly this regulation would be altered in Fy (a-b-) subjects (Anstee, 2010). The data provide evidence that SCD patients with the Fy (a-b-) phenotype are more susceptible to chronic organ damage and proteinuria than SCD patients of normal Fy phenotype and are consistent with such an hypothesis (Afenyi-Annan *et al*., 2008). A further consequence of selection for the Fy (a-b-) phenotype in Africa may be to alter the kinetics of HIV-1 infection in those with this phenotype. Several HIV-1 strains bind to Duffy on normal red cells, facilitating the transfer of HIV-1 to its target cells (CD4 + /CCR5 + T lymphocytes) with 5- to 12-fold greater efficiency than Fy (a-b-) red cells. 97 He et al 97 calculate that patients with the Fy(a-b-) phenotype have a 40% greater likelihood of acquiring HIV than those lacking the phenotype; however, the disease, once acquired, has a slower progression than in infected patients of normal Fy type. They conclude that these differences are related to loss of competition for binding HIV-1 between plasma chemokine CCR5 and Duffy on red cells in Fy (a-b-) subjects and consequent changes in the inflammatory state those infected. The findings of this study have been contested by Walley *et al*. (2009) who used different methodology to analyze a different cohort of HIV+ and HIV- African American subjects and found no association between Fy genotype and progression to AIDS or risk for HIV acquisition. They also point out that the number of HIV patents used by was much smaller (227 vs 814) and suggest this difference may be a major factor affecting the analysis.

2.2.4 Different strains of *P. falciparum* use different blood group proteins as receptors:

The dual availability of in vitro culture systems to study the invasion of human red cells by *P. falciparum* and well-characterized rare blood group phenotypes made it possible to identify red cell receptors used by different parasite strains. Early studies on cells lacking glycophorin A (Ena-cells) and glycophorin B (S-s-cells) provided evidence that these sialic acid-rich red cell-surface glycoproteins were parasite receptors and these observations have been confirmed. Glycophorins C (GPC) and D (Ge- red cells) are also receptors for some strains of *P. falciparum.* Glycophorins are major proteins at the red cell surface. Glycophorin A (GPA) and the major anion transport protein (AE1, Band 3) with approximately 10,000000 copies/red cell are the most abundant red cell surface proteins with glycophorins B, C, and D together accounting for a further 450 000 copies per red cell. Perhaps surprisingly, there is little experimental evidence to suggest selection against the expression of GPA has occurred in response to *P. falciparum* infection. Red cells from patients having the hybrid GPA-GPB protein Dantu, which is common in certain parts of Africa, are reported to resist invasion, and it has been suggested that elevated expression of Band 3 occurring in patients with the GPB-GPA-GPB MiIII protein common in South East Asia may be relevant to malaria survival. The importance of sialic acid on GPA in forming a receptor for *P .falciparum* suggests that red cells expressing glycosylation variants of GPA found commonly in Africans in which N-acetyl D-glucosamine is present in some of the sialic acid-rich O-glycans at the N-terminus (patients with the M 1 antigen) may be relevant to one's susceptibility to malaria (Anstee, 2010). In contrast to the situation with GPA, subjects lacking glycophorin B are found in high frequency in central Africa. Patients with red cells lacking GPC and glycophorin D (Ge-, Leach phenotype) are very rare, but those with Ge- red cells having an altered GPC resulting from deletion of exon 3 of GYPC are

common in Melanesians, most notably in Papua New Guinea, and the resulting phenotype provides protection against *P .falciparum*. Clearly, different strains of *P. falciparum* target glycophorins associated with one or other of the membrane complexes, providing key cytoskeletal linkages maintaining the stability of the red cell membrane and selection, resulting in loss or alteration of glycophorins in either of these sites confers a survival advantage (Anstee, 2010). Melanesians also exhibit another example of selection, affording protection against cerebral malaria, a phenotype known as South East Asian ovalocytosis. South East Asian ovalocytosis cells, as the name implies, have an abnormal shape. They are also characterized by weakened expression of a large number of blood group antigens, including antigens found on Band 3, GPA, and the Rh blood group proteins. In this case selection favors the heterozygote. Heterozygotes inherit a normal band 3 gene together with a mutant inactive band 3 gene resulting from a deletion, causing a loss of 9 amino acids at the point at which the cytoplasmic N-terminal domain enters the cytosolic face of the lipid bilayer. Homozygous inheritance of this mutation would result in total Band 3 deficiency. Because Band 3 is essential for respiration (Cl/HCO 3 exchange) and for maintaining the integrity of the red cell membrane, it must be assumed in evolutionary terms that such an inheritance is incompatible with survival. Complement receptor carries antigens of the Knops blood group system. CR1 expression is very variable between patients and red cells expressing fewer than 100 copies CR1 per cell show reduced rosetting with *P.falciparum* strain R29R, as do red cells expressing the Sl a-blood group phenotype. The Sl a-phenotype, which results from a single nucleotide polymorphism (R1601G) in long homologous repeat D, occurs in only 1% of the white population but reaches 70% in Malians (Anstee, 2010).

2.3 Malaria and blood groups:

ABO blood group system is genetically controlled and pro-protein of various ABO groups differ significantly in different population and ethnic groups. Thus, any national or international study reporting association of ABO groups with a disease must use population frequency of ABO groups as the base for comparison. A, B, H antigens synthesis involves addition of sugars to paragloboside, N-acetyls galactose amine is specific for 'A', D galactose for 'B' and L fructose for 'H'. 'O' group erythrocytes are less prone to form rosettes with *P.falciparum* parasite infected RBC because of the reduced cytoadhesion and rosette formation with parasite. Hence 'O' group individuals have reduced risk of serve malaria. However, 'O' group shows significant association with placental *P.falciparum* malaria infection. Akanbi *et al*. (2010) have observed that 'A' group has more parasite density than 'B' and 'O'. 'O' group red cells have minimum density. Fry *et al*. (2008) have tested three African populations for ABO alleles by molecular methods. They observed that haplotype in 'O' and none 'O' individual are different and might lead to malaria susceptibility of population (Fry *et al*., 2008).

2.3.1 Severity of *falciparum* malaria and blood groups:

In view of a heavy burden placed on human health due to malaria, a good many investigations have been conducted to find out whether or not ABO blood groups antigens are associated with susceptibility, resistance, or severity of *P. falciparum* malaria. Nonetheless, these studies have reported contradictory results. Some studies reported the absence of significant association between *P. falciparum* (prevalence, parasitaemia or antibody titer) and ABO antigens. On the other hand, other studies have shown that high frequency of malaria episodes has been observed among blood group ‘A’ individuals as compared with other blood groups individuals. Large numbers of severe malaria cases were also reported among blood group ‘A’ individuals. Furthermore, Pathirana *et al*. (2005) observed low parasitaemia and uncomplicated malaria cases among

blood group ‘O’ individuals. Variations in reports on the association of ABO blood groups and disease progression of *P. falciparum* malaria show the complexity of the interaction between the parasites and host immune responses. In addition, studies have shown the impact of other red blood cells (RBC) polymorphisms including haemoglobin (Hb) abnormalities such as HbS, HbC, thalassemia and deficiency in erythrocyte complement receptor (CR) or glucose-6-phosphate dehydrogenas on *P. falciparum* malaria susceptibility and severity. This makes it difficult to make a clear analysis on the association of ABO blood groups and *P. falciparum* because so far most of the study designs have been conducted in vivo (Zurihan *et al*., 2011). Rosetting may be a parasite virulence factor because it causes micro-vascular obstruction, which is thought to be a key process in the pathogenesis of severe malaria. In an ex vivo model, *P. falciparum* rosettes were disrupted by high shear forces in the arterial side of the circulation but reformed in the post-capillary venules by adhesion of uninfected erythrocytes onto infected erythrocytes that were bound to endothelial cells. This combination of rosetting and cytoadherence occurring simultaneously resulted in rosetting parasites causing greater obstruction to micro-vascular blood flow than isogenic cytoadherent non-rosetting parasites. The ABO determinants are present on endothelial cells and platelets as well as erythrocytes, and it seems likely that parasite isolates that bind to A or B determinants on erythrocytes to form rosettes may also bind A or B antigens on other cell types, which could enhance sequestration and increase pathogenic potential, blood group O protects against severe malaria in a matched case control study of Malian children, and provided by evidence for a similar protective effect of group O in Kenya. Statistical analysis of the Malian study indicates a significant interaction between the host ABO blood group and parasite rosette frequency that provides strong evidence to support the hypothesis that group O protects by the mechanism of reduced rosetting and sequestration. These findings indicate that blood group O provides a further

example of an erythrocyte polymorphism that, similarly to CR1 deficiency, α-thalassemia, and HbC, is able to reduce the adhesion potential of *P. falciparum*-infected erythrocytes and consequently modify the virulence of the parasite (Rowe *et al*., 2007).

Chapter 3

Materials and methods

3.1 Study design:

It is a cross-sectional hospital based study.

3.2 Study area:

The study was carried out in Giad and Al Gadeed hospitals, Gezira state- Sudan.

3.3 Study population:

For purpose of this study, patients suspected of having malaria were selected from different hospitals and health care centers in different endemic areas mentioned above.

3.4 Study duration:

This study was carried out in a period from June 2017 to September 2018.

3.5 Sample size:

The study was conducted on 280 blood samples, 140 from Giad hospital and 140 from Al Gadeed Al Thawra hospital.

3.6 Sampling:

Blood samples were collected from all individuals under the study. Collections were taken randomly by using simple random sampling method. Each hospital was chosen randomly and entirely by chance such that each hospital had the same probability of being chosen at any stage during the sampling process. Blood samples were examined by stained thick blood films for detection of malaria parasites and stained thin blood film for identification of species. Also, samples were examined for ABO blood groups.

3.7 Data collection:

The primary data were collected by using designed questionnaire (appendix) to obtain information that helped in the study.

3.8 Collection of blood samples:

Capillary or venous blood samples were collected.

3.8.1 Collection of capillary blood:

With cotton wool dipped in 70% alcohol, the tip of the third finger was cleaned, and with sterile lancet, finger was pricked firmly and rapidly. The first drop of blood was wiped (Anonymous, 1992).

3.8.2 Collection of venous blood:

For venous blood, tourniquet was tied around the right or left elbow and then cotton wool dipped in 70% alcohol was used to clean the arm on a visible vein. The needle was then introduced to the vein and 2.5 ml of blood collected and placed in a blood container with EDTA anticoagulant (Baird *et al*., 1992).

3.9 Preparation of blood smears:

3.9.1 Preparation of thick 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 (Anonymous, 1992).

3.9.2 Preparation of thin blood smears:

A drop of blood was placed on the middle of clean and dry slide and by edge of another slide (called spreader) 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 (Anonymous, 1992).

3.10 Staining of blood films:

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.

3.11 Examination of blood films:

The slides were examined using light microscope (Olympus x100 oil immersion lenses). The number of parasites were counted and reported by using the following grading as described by Anonymous (1992):

- l- 10 parasites per 100 thick film fields +
- 11- 100 parasites per 100 thick film fields ++
- 10 parasites per thick film field +++
- 11- 100 parasites per thick film field ++++

3.12 Procedure of ABO blood groups:

With a grease pencil two circle were drawn on clean and dry slide, and labeled one (A) and another (B), and on another slide circle was drawn and labeled (D), then a drop of blood was placed on each circle, then to the circle (A) drop of anti-serum A was added, drop of anti-serum B also was added to circle (B), and drop of anti-serum D was added to circle (D) on the another slide. Then each suspension was mixed with different wood stick (Dietze *et al*., 1995).

3.13 Interpretation of ABO blood groups:

The interpretation of ABO blood was group as follows as described by Dietze *et al*. (1995):

Agglutination on (A) circle and no agglutination on (B) circle mean the ABO blood group is A.

- Agglutination on (B) circle and no agglutination on (A) circle mean the ABO blood group is B.
- Agglutination on Both (A) circle and (B) circle mean the ABO blood group is AB.
- No agglutination on both (A) circle and (B) circle mean the ABO blood group is O.
- Agglutination on (D) circle means the Rhuses factor (Rh-factor) is positive (+ve).

- No agglutination on (D) circle means the Rhuses factor (Rh-factor) is negative (-ve).

3.14 Ethical consideration:

The study adopted was approved by College of Medical Laboratory Science-Sudan University of Science and Technology and permission was taken from all individuals or their guardians before being included in the study. Each individual was informed on the nature of the study.

Chapter 4

Results

4.1 The overall prevalence rate of malaria in all hospitals investigated:

Out of the 280 blood samples examined in the 2 hospitals investigated, 100 samples were found positive for malaria infection. This constituted an overall prevalence rate of 35.7% (table 4.1).

Table (4.1): The overall prevalence rate of malaria in all hospitals investigated

No. of sample examined	No. positive	Percentage (%)
280	100	35.7%

4.2 The prevalence rates of malaria in each hospital investigated:

The prevalence rates reported in Gaid and Al Gadeed Al Thora hospitals were 25.7 % and 45.7% respectively (table 4.2). The difference in rates was found to be statistically highly significant at p. value= 0.000.

Table (4.2): The prevalence rates of malaria in each hospital investigated

Hospitals	No. of sample examined	No. positive	Percentage (%)
Giad	140	36	25.7%
Al Gadeed Al Thora	140	64	45.7%
Total	280	100	35.7

4.3 The prevalence rates of malaria infection in Giad hospital according to gender:

The results showed that the highest prevalence rate (30%) was reported among females, while males reported prevalence rate (18%) (table 4.3). The difference in rates was found to be statistically insignificant at p. value= 0.293

Table (4.3): The prevalence rates of malaria infection in Giad hospital according to gender

Gender	No. of sample examined	No. positive	Percentage (%)
Males	50	9	18%
Females	90	27	30%
Total	140	36	25.7%

4.4 The prevalence rates of malaria infection in Giad hospital according to age groups:

The highest prevalence rate (40%) was reported among the 16-30 years old and the lowest rate (16%) was reported among the 46-60 years old (table 4.4). The difference in rates between all age groups was found to be statistically significant at p. value= 0.006.

Table (4.4): The prevalence rates of malaria infection in Giad hospital according to age groups

Age group (years)	No. of sample examined	No. positive	Percentage (%)
1-15	55	14	25.4%
16-30	20	8	40%
31-45	40	10	25%
46-60	25	4	16%
Total	140	36	25.7%

4.5 The prevalence rates of malaria infection in Giad hospital according to blood groups:

The investigation revealed that the highest prevalence rate (31.2%) was reported among the A-ve blood group and the lowest prevalence rate (7.1%) was reported among the B-ve blood group (table 4.5). The difference in rates was found to be statistically highly significant at p. value= 0.000

Table (4.5): The prevalence rates of malaria infection according to blood groups in Giad hospital

Blood groups	No. of sample examined	No. positive	Percentage (%)
A+ve	35	10	28.5%
A-ve	16	5	31.2%
B+ve	24	6	25%
B-ve	14	1	7.1%
O+ve	43	13	30.2%
O-ve	6	1	16.6%
AB+ve	2	0	0%
AB-ve	0	0	0%
Total	140	36	25.7%

4.6 The different levels of parasitaemia for different blood groups in Giad hospital:

High parasitaemia (++++) was strictly confined to the A+ve blood group with a rate of 17.1% while severe parasitaemia (+++) was more evident with the O-ve blood group (16.6%). Low parasitaemia (+) was more evident with the O+ve blood group (4.7%) while moderate parasitaemia (++) was mostly observed with the O +ve blood group (13.9%) (table 4.6). The difference in rates between parasitaemia and blood groups was found to be statistically highly significant at p. value=.0.000.

Table (4.6): The different levels of parasitaemia for different blood groups in Giad hospital

Blood groups	No. of sample examined	No. positive	+ (%)	++ (%)	+++ (%)	++++ (%)
A+ve	35	10	0 (0%)	2 (5.7%)	2 (5.7%)	6 (17.1%)
A-ve	16	5	0 (0%)	1 (6.2%)	2 (12.5%)	2 (12.5%)
B+ve	24	6	0 (0%)	2 (8.3%)	1 (4.2%)	3 (12.5%)
B-ve	14	1	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)
O+ve	43	13	2 (4.7%)	6 (13.9%)	4 (9.3%)	1 (2.3%)
O-ve	6	1	0 (0%)	0 (0%)	1 (16.6%)	0 (0%)
AB+ve	2	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
AB-ve	0	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	140	36	25.7%			

4.7 The different blood groups for different species in Giad hospital:

The highest prevalence rate (22.8%) was reported for *P. falciparum* for A+ve blood group and rate (16.2%) for *P. vivax* for A-ve while the rate (6.2%) was reported for mixed infection for A-ve (table 4.7).The difference in rates was found to be statistically insignificant at p. value= 0.375.

Table (4.7): The different blood groups for different species in Giad hospital

Blood groups	No. of sample examined	No. positive	PF (%)	PV (%)	PV+PF (%)
A+ve	35	10	8 (22.8%)	2 (5.7%)	0 (0%)
A-ve	16	5	3 (18.7%)	1 (6.2%)	1 (6.2%)
B+ve	24	6	4 (16.6%)	2 (8.3%)	0 (0%)
B-ve	14	1	1 (7.1%)	0 (0%)	0 (0%)
O+ve	43	13	6 (13.9%)	7 (16.2%)	0 (0%)
O-ve	6	1	1 (16.6%)	0 (0%)	0 (0%)
AB+ve	2	0	0 (0%)	0 (0%)	0 (0%)
AB-ve	0	0	0 (0%)	0 (0%)	0 (0%)
Total	140	36	25.7		

4.8 The prevalence rates of malaria infection in Al Gadeed Al Thawra hospital according to gender:

The results showed that highest prevalence rate (50%) was reported among males while females reported (42.5%) prevalence rate (table 4.8). The difference in rates was found to be statistically insignificant at p. value= 0.292.

Table (4.8): The prevalence rates of malaria infection in Al Gadeed Al Thawra hospital according to gender

Gender	No. of sample examined	No. positive	Percentage (%)
Male	60	30	50%
Female	80	34	42.5%
Total	140	64	45.7%

4.9 The prevalence rates of malaria infection in Al Gadeed Al Thawra hospital according to age groups:

The highest prevalence (71.1%) was reported among 1-15 years old and lowest rate (21.4%) was reported among 46-60 years old (table 4.9).The difference in

rates between all age groups was found to be statistically highly significant at p. value= 0.000 .

Table (4.9): The prevalence rates of malaria infection in Al Gadeed Al-Thawra hospital according to age groups

Age group (years)	No. of sample examined	No. positive	Percentage (%)
1-15	45	32	71.1%
16-30	35	14	40%
31-45	32	12	37.5%
46-60	28	6	21.4%
Total	140	64	45.7%

4.10 The prevalence rates of malaria infection according to blood groups in Al Gadeed Al Thawra hospital:

The investigation revealed that the highest prevalence rate (57%) was reported among the O-ve blood group and lowest prevalence rate (20%) was reported among the AB-ve blood groups (table 4.10). The difference in rates was found to be statistically highly significant at p. value= 0.000

Table (4.10): The prevalence rates of malaria infection according to blood groups in Al Gadeed Al Thawra hospital

Blood groups	No. of sample examined	No. positive	Percentage (%)
A+ve	44	24	54.5%
A-ve	17	7	41.1%
B+ve	19	6	31%
B-ve	9	3	33%
O+ve	32	16	50%
O-ve	7	4	57%
AB+ve	7	3	43%
AB-ve	5	1	20%
Total	140	64	45.7%

4.11 The different levels of parasitaemia for different blood groups in Al-Gadeed Al Thawra hospital:

High parasitaemia (++++) was strictly confined to the A+ve blood group with rate of 34% while severe parasitaemia (+++) was more evident with the B-ve blood group (22.2%). Low parasitaemia (+) was more evident with the O+ve blood group (25%) while moderate parasitaemia (++) was mostly observed with the O-ve blood group (42.8%) (table 4.11). The difference in rates between parasitaemia and blood groups was found to be statistically highly significant at p. value=.0.000

Table (4.11): The different levels of parasitaemia for different blood groups in Al Gadeed Al Thawra hospital

Blood groups	No. of sample examined	No. positive	+ (%)	++ (%)	+++ (%)	++++ (%)
A+ve	44	24	1 (2.2%)	1(2.2%)	7 (15.9%)	15 (34%)
A-ve	17	7	0 (0%)	0 (0%)	3 (17.6%)	4 (23.5%)
B+ve	19	6	0 (0%)	0 (0%)	3 (15.7%)	3 (15.7%)
B-ve	9	3	0 (0%)	0 (0%)	2 (22.2%)	1 (11.1%)
O+ve	32	16	8 (25%)	8 (25%)	0 (0%)	0 (0%)
O-ve	7	4	1 (14.2%)	3 (42.8%)	0 (0%)	0 (0%)
AB+ve	7	3	0 (0%)	0 (0%)	1 (14.2%)	2 (28.5%)
AB-ve	5	1	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Total	140	64	45.7			

4.12 The different blood groups for different species in Al Gadeed Al Thawra hospital:

The highest prevalence rate (41.1%) was reported for *P. falciparum* for A-ve blood group and rate (31.2%) for *P. vivax* for O+ve while the rate (6.8%) was reported for mixed infection for A+ve (table 4.12).The difference in rates was found to be statistically insignificant at p. value= 0.068.

Table (4.12): The different blood groups for different species in Al Gadeed Al Thawra hospital

Blood groups	No. of sample examined	No. positive	PF (%)	PV (%)	PV+PF (%)
A+ve	44	24	13(29.5%)	8 (18.1%)	3 (6.8%)
A-ve	17	7	7 (41.1%)	0 (0%)	0 (0%)
B+ve	19	6	5 (26.3%)	1 (5.2%)	0 (0%)
B-ve	9	3	1 (11.1%)	2 (22.2%)	0 (0%)
O+ve	32	16	5 (15.6%)	10 (31.2%)	1 (3.1%)
O-ve	7	4	2 (28.5%)	2 (28.5%)	0 (0%)
AB+ve	7	3	2 (28.5%)	1 (14.2%)	0 (0%)
AB-ve	5	1	1 (20%)	0 (0%)	0 (0%)
Total	140	64	45.7		

Chapter 5

Discussion, conclusion and recommendations

5.1 Discussion:

From the results, it was obvious that the overall prevalence rate in the 2 hospitals investigated was relatively high (35.7%). This rate was found to be highest than the 27.5% rate (reported by Abdallah (2010) in El-Duem and Abd-Alla *et al*. (2016) in Khartoum, Singa and Al Genaid, however it was lower than the 66% rate reported by Ibrahim (2008) in Soba hospital- Khartoum state. From the investigation in Giad, the highest rate (40%) was reported with the age group 16-30 years old. This rate was higher than the 26.7% rate reported by Abdallah (2010) in the age group less than 10 years old; however, it was lower than the 60% and 41.7% rate reported by Ibrahim (2008) and Abd Alla *et al.* (2016) respectively for the age group less than 10 years old. As far as gender was concerned, the results showed that females reported the highest rate (30%), while males reported a 18% rate. These rates were closer to the rates reported by Abdallah (2010) in Elduem for males and females (26.1 % and 28.6 % respectively), however, these findings were in disagreement with the findings of Abd Alla *et al.* (2016) in Khartoum, Singa and Al Genaid for males and females (31.2 % and 24.7 % respectively) and Ibrahim (2008) for males and females (53.3 % and 26 % respectively). From the results obtained from the present study, it was clear that the high rate (31.2%) of malaria infection was reported with the A-ve blood group while the lower rate (7.1%) was reported with B-ve. This rate of infection was in agreement with the finding of Anstee (2010) and in disagreement with the finding of Akhingbe *et al*. (2011) and Abd Alla *et al*. (2016) who reported that subjects with group O+ve had a higher prevalence of malaria infection. From the results, high parasitaemia (++++) was reported with the A+ve blood group with a rate of 17.1% while the low parasitaemia (+) was reported with the O+ve with a rate of 4.7%. This finding was in agreement with the finding of Igbeneghu *et al*. (2012) who reported that O individuals appeared

to be the most protected against high parasite density while A individuals were more likely to experience high parasite density. The highest prevalence rate (22.8%) was reported for *P. falciparum* for A+ve while the lowest rate (6.2%) was reported for mixed infections with *P. falciparum* and *P. vivax* species for A-ve. This rate was higher than the rate reported by Abdallah (2010) who reported a 14% rate for *P. falciparum* and lower than the 8% rate for mixed infections for O+ve group.

From the investigation in Al Gadeed Al Thawra hospital, the highest prevalence (71.1%) was reported among 1-15 years old and lowest rate (21.4%) was reported among 46-60 years old. This rate was higher than the 54% rate reported by Abdallah (2010) in the same age group. However, it was lower than the 89% rate reported by Ibrahim (2008) for the same age group. As for as gender was concerned, our results showed that the males reported the highest rate (50%) while the females reported a 42.5% rate, these rates were closer to the rates reported by Ibrahim (2008) (53.3% and 36% respectively). The results revealed that the highest prevalence rate (57%) was reported among the O-ve blood group and the lowest prevalence rate (20%) was reported among the AB-ve blood groups. This rate of infection was in agreement with the finding of Tekeste and Petros (2010) in Ethiopia who reported that the higher rate (21.4%) for O-ve group. From the results, it was clear that the highest rate of malaria parasitaemia (++++) was reported with A+ve blood group with a 34% rate while the low parasitaemia (+) was more evident with the O+ve blood group with a 25% rate. The highest prevalence rate (41.1%) was reported for *P. falciparum* for A-ve blood group and 31.2% rate for *P. vivax* for O+ve while the 6.8% rate was reported for mixed infections for A+ve. These rates were higher than the 14% rate for *P. falciparum* and lower than the 8% rate for mixed infections for O+ve group reported by Abdallah (2010).

5.2 Conclusion:

The study concluded that overall prevalence rate in the 2 hospitals investigated was relatively high (35.7%). High parasitaemia was most likely to occur in subjects with blood groups A and B, however, individuals with O blood group were less likely to exhibit high parasitaemia. Gender does not have any role in the establishment of the disease.

5.3 Recommendations:

- More efforts should be exerted by the health authorities to further control and irradiate the disease.
- Future work should consider the haemoglobin genotypes in addition to ABO blood grouping to reveal the exact picture of the association.
- More research should be conducted to elucidate the picture on the association between malaria infection and ABO blood group.
- When applying similar studies, increasing the sample size is a necessity to have a true picture of the association.

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Appendix

Sudan University of Science and Technology

College of Graduate Studies

M.Sc. in Parasitology and Medical Entomology

Questionnaire form

- Date: ……..

- ID: ……..

- Giad hospital ☐ - Al Gadeed Al Thawra hospital ☐

General information:

- Name: ……..

- Gender: ……

- Age: ……

Laboratory investigations:

- Laboratory results of malaria:

Blood film:

Thick +ve ☐ -ve ☐

Density: + ☐ ++ ☐ +++ ☐ ++++ ☐

Thin

Species: …… Stage (s): ……

Laboratory results of ABO blood groups:

Blood types:

A+ve ☐ A-ve ☐ B+ve ☐ B-ve ☐

O+ve ☐ O-ve ☐ AB+ve ☐ AB-ve ☐