Sudan University of Science and Technology

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Association of ABO and Rh blood groups with Malaria infection in 24 Algorashi Area in Aljazeera State - Sudan

ارتباط مجموعات الدم ABO و Rh بالإصابة بمرض الملاريا بمنطقة 24 القرشي بولاية الجزيرة بالسودان

A dissertation submitted in partial fulfillments of the requirements for the MSc in Haematology and Immunohaemtology

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بسم الله الرحمن الرحيم

قال تعالى :

إِنَّ اللَّـهَ لَا يَسْتَحْيِي أَن يَضْرِبَ مَثَلًا مَّا بَعُوضَةً فَمَا فَوْقَهَا ۚ فَأَمَّا الَّذِينَ آمَنُوا فَيَعْلَمُونَ أَنَّهُ الْحَقُّ مِن رَّبِّهِمْ ۖ وَأَمَّا الَّذِينَ كَفَرُوا فَيَقُولُونَ مَاذَا أَرَادَ اللَّـهُ بِهَـٰذَا مَثَلًا ۘ يُضِلُّ بِهِ كَثِيرًا وَيَهْدِي بِهِ كَثِيرًا ۚ وَمَا يُضِلُّ بِهِ إِلَّا الْفَاسِقِينَ ﴿٢٦﴾

صدق الله العظيم

سورة البقرة الآية 26

Dedication

To

my father soul, dear mother , sisters and brothers

Soha

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Abstract

Objectives: The study was aimed to detect possible association between the ABO blood groups and the malaria diseasein a cross- sectioned group sample in the endemic area in Central Sudan .

Materials and Methods: during April 2017, a group of 150 patient visited *24 Algurashi*hospital – Aljazeera State- central Sudan - and referred to the laboratory for malaria investigations. Malaria positive patients' samples(114 out of 150) were correlated with the blood grouping of each patient, considering their ages and gender.

Results: A total of 150 patients were included in the study, of which 114 cases were positive for *Plasmodium falciparum*. The number of adults affected (n= 52) was nearly equal to the number of children (n = 48).Among the adults, this study showed different age groups had no significant correlation with incidence of malaria.The prevalence was higher among males (53.51%) than females (46.49%) and this difference was not statistically significant (p value>0.05). The results of the blood groups of the infected patients (n = 114) showed 28patients had 'A+' group, 14patients'B+' group, 64patients'O' group and 3 was 'AB+' group.It wasobserved that 'O' group had an advantage over other the groups (52.83%). The majority of smears showed (one +) parasitemia of malaria.

Conclusion: All age groups and both genders were affected.'O+' blood group had an advantage over the other three blood groups. Based on literature and the results of this study. In the present study, although malaria was more frequent among patients classified with blood group O positive Rh, an association between blood group and malaria could not be established(p value>0.05).This study supports the hypothesis that group 'O' individuals may be more associated with Malaria infection. Younger children were more susceptiblethan adultsto malaria infection. The study had recommended that it is unnecessary to test blood groupfor Malaria infected patients, and to carry further studies about association between Malaria and blood groups.

مستخلص الدراسة

أهداف الدراسة: أجريت هذه الدراسة للربط بين فصيلة الدم و الإصابة بمرض الملاريا، في دراسة لعينة محددة أخذت من منطقة 24 القرشي بأواسط السودان حيث تنتشر الإصابة بالملاريا.

طريقة البحث: في ابريل 2017، تمت دراسة عدد 150 عينة من أشخاص تم تشخيصهم بمستشفى 24 القرشي– ولاية الجزيرة –أواسط السودان - و تحويلهم للفحص المعملي للملاريا. اظهر الفحص بواسطة صبغة جمسا وجود طفيل الملاريا في 114 من هذه العينات. ثم تمت قراءة فصيلة الدم لنفس العينات، و تم الربط بين وجود الملاريا و فصيلة الدم لكل مريض مع ذكر عمر المريض و جنسه.

النتائج: أظهرت الفحوصات 114 فحص ايجابي للملاريا – بلازموديوم فالسيبارممن العينة العامة (150 مريض)، فان 114 اظهروا. عدد المصابين البالغين (52 شخص) يقارب عدد الأطفال المصابين (48 طفل). بين هؤلاء البالغين، أظهرت الدراسة عدم وجود علاقة ذات دلالة إحصائية بين المجموعات العمرية المختلفة و الإصابة بمرض الملاريا.و كانتالاصابة بالمرض اكثر انتشارا بين الرجال (53.51%) من النساء (46.49%) و لكن الفرق بينهما ليس ذو دلالة إحصائية (p >0.05). نتائج فحص فصائل الدم للأشخاص المصابين بالملاريا (114 شخص) كانت كالتالي: 28 مريض من فصيلة (A+) ، 9 مرضى من فصيلة (B+) ، 64 مريض من فصيلة (O+)، و 2 مريض من فصيلة (AB+). يلاحظ ان فصيلة الدم O+ لها السيادة على الفصائل الأخرى بنسبة (52.83%) بالنسبة للقابلية للإصابة بمرض الملاريا. الغالبية العظمى من العينات أظهرت كثافة (صليب واحد " +") من الطفيل.

الخلاصة: كل المجموعات العمرية و كلا الجنسين معرضون للإصابة بالملاريا. نسبة فصيلة الدم O+ فاقت الفصائل الأخرى بين المصابين بالملاريا، هذه الحقيقة أكدتها دراستنا هذه و دراسات أخرى تم عرضها. رغم ان هذه الدراسة قد أثبتت ان الملاريا أكثر انتشارا لدى المرضى ذوي الفصيلة O+ ، لكن لم تتمكن الدراسة من تحديد دقيق للربط بين فصيلة الدم و الإصابة بالملاريا(p value>0.05).دعمت هذه الدراسة فرضية ان الأشخاصأصحاب فصيلة الدم O+ قد يكونون أكثر ارتباطا للإصابة بالملاريا. الأطفال الصغار أكثر عرضة للإصابة بالملاريا من البالغين. أوصت الدراسة بعدم ضرورة فحص فصيلة الدم لدى مرضى الملاريا لانعدام العلاقة بينهما، كما أوصت بإجراء المزيد من الدراسات حول العلاقة بين فصيلة الدم و الإصابة بمرض الملاريا.

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Abbreviations

ABO	A blood grouping system
BF	Blood film for malaria test
RBCs	Red Blood Cells
WBC	White Blood Cells
P. falciparum	Plasmodium . falciparum
SPSS	Statistical package Social Sciences

Chapter One

Introduction and Literature review

1.1 Introduction

Malaria is considered one of the most important tropical illnesses in public health causing millions of infections and deaths each year. Many studies have tried to establish an association between the severe form of the disease and the ABO-blood group type. In countries with large endemic zones for malaria there are not enough studies or statistic data about this possible association (Uneke 2006).

Malaria remains the most complex and overwhelming health problem facing humanity especially in the vast majority of tropical and subtropical regions of the world with 300 to 500 million cases and 2 to 3 million deaths per year (WHO 2000).

The disease caused by the intracellular protozoan parasite of the genus Plasmodium invades and multiplies in the liver and red blood cells (RBCs) during its life cycle in man. Malaria is a scourge of nations of Africa, India, Southeast Asia, and South America (WHO 2003).

Malaria is considered a worldwide major public health problem (Botero and Restrepo 1998), as it is the most important parasite disease affecting humans and causing millions of deaths each year (White and Breman 2005). In tropical and subtropical regions of the world, it is estimated that malaria is responsible for 300 to 500 millions of infections and 2 to 3 millions of deaths per year (WHO 2000); being considered as one of the main health problems in regions like Africa, India, Southeast of Asia, and South America (WHO 2003). Despite the remarkable progress in prevention, the global tally of malaria in 2015 was 212 million new cases

and 429 000 deaths. Across Africa, millions of people still lack access to the tools they need to prevent and treat the disease. (WHO 2016).

There is increasing evidence that Plasmodium *falciparum* malaria is influenced by ABO blood group but the extent of association between both is yet to be well defined. There were apparent discrepancies and contradictions in the studies as some reported significant association between both while others observed no significant association. This outcome may reflect the complex interaction between P. *falciparum* malaria and the host immune responses. However, findings from all studies reviewed suggested that individuals of blood group O are relatively resistant to severe disease caused by *P. falciparum* infection.

It was established that parasitized erythrocytes form rosettes more readily with red blood cells (RBCs) of A, B, or AB groups than with blood group O and this parasite-triggered RBC rosette formation is associated with the severity of clinical disease and with the development of cerebral malaria. (Uneke2007).

The sequence of RBC invasion, which is well established for P. falciparum infection, is probably similar for the three other species of the malaria parasite (Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae) and therefore establishes the direct relationship between malaria and the RBC (Weatherall 2008).

Significant associations of malaria with several RBC polymorphisms (hemoglobin, glucose-6-phosphate dehydrogenase, ABO blood group, and Duffy systems) were detected in many populations, especially those living under hyperendemic conditions in the Old World (Barragan et al. 2000).

Clinical severity, rather than incidence or prevalence of detectable parasitemia, is a more relevant outcome to assess ABO group and survival. Studies reporting clinical features such as cerebral malaria carry more weight than those reporting only laboratory markers such as percent parasitemia, because the latter does not always predict survival. Among those with a well-developed humoral immunity, there is little correlation between high circulating parasitemia and severity of illness. *Plasmodium falciparum* infection increases the serum levels of IgM and IgG antibodies, also IgE in individuals living in endemic areas. The association of high anti PF IgE levels with a reduced risk of developing clinical malaria suggests the involvement of IgE in protection (Duarte 2007).

Malaria is known to have affected many erythrocyte genes, including those concerned with globin synthesis, membrane proteins, and RBC enzymes. Given the importance of RBCs in malaria, an influence on genes encoding the most abundant antigens on the RBC membrane such as blood groups is expected . It is also difficult to dissociate the role played by ABO sugars from the contribution of other glycosylated adhesion molecules. The study of *Vector* including both *P. falciparum* and *P. vivax* infections provides supporting evidence in favour of an effect of ABO group on disease severity as 'O' group provides advantage over non 'O' groups. (Deepa *et.al* 2011).

The association of genetic markers with malaria has been the subject of numerous investigations, since the protection afforded by sickle-cell hemoglobin against infection by *falciparum* malaria parasite. A broad range of available evidence suggests that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *Plasmodium*

falciparum infection. Clinical reports of ABO blood groups and *P. falciparum* infection, reveals a correlation between disease severity and ABO groups. However, several studies undertaken have been unable to link ABO blood groups to the incidence of malaria or to the repeat attacks of malaria (Singh 1995).

1.2 Literature review

1.2.1 Blood grouping

Blood is typed, or classified, according to the presence or absence of certain markers (antigens) found on red blood cells and in the plasma that allow the body to recognize blood as its own. If another blood type is introduced, the immune system recognizes it as foreign and attacks it, resulting in a transfusion reaction. (Dean, Laura 2005).

1.2.1 .1 History of discoveries of the blood types

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who identified the O, A, and B blood types in 1900 . Landsteiner originally described the O blood type as type "C", and in parts of Europe it is rendered as "0" (zero), signifying the lack of A or B antigen. Landsteiner was awarded the Nobel Prize in Physiology or Medicine in 1930 for his work. Alfred von Decastello and Adriano Sturli discovered the fourth type, AB, in1902 (Hans Peter 2003) .

The precursor to the ABO blood group antigens, present in people of all common blood types, is called the H antigen. (Dean, Laura 2005).

The ABO system consists of A, B, AB, and O blood types. People with type A have antibodies in the blood against type B. People with type B have antibodies in the blood against type A. People with AB have no anti-A or anti-B antibodies. People with type O have both anti-A and anti-B antibodies. People with type AB blood are called universal recipients, because they can receive any of the ABO types. People with type O blood are called universal donors, because their blood can be given to people with any of the ABO types. Mismatches with the ABO and Rh blood types are responsible for the most serious, sometimes life-threatening,

transfusion reactions. But these types of reactions are rare. (Stryer L., 2002).

For every 1 million units of blood transfused, getting the wrong blood type happens, at the most, 4 times. Transfusion with the wrong blood type can cause a severe reaction that may be life-threatening. ABO blood group refers to a system of carbohydrate antigens expressed on human erythrocytes and other human cells. The “A” and “B” antigens on erythrocytes are trisaccharides (Daniels, 2005) .

All erythrocytes possess an “H” disaccharide on their surfaces (except the rare Bombay phenotype, which has no ABO antigens). Individuals with blood groups “A” and “B” have the “A” and “B” antigens, respectively, together with the “H” antigen. Blood group “AB” individuals have both “A” and “B” antigens together with the “H.” Blood group “O” individuals, however, have neither “A” nor “B” antigens but “H.” (Loscertales *et al*, 2007).

1.2.1 .2 Rh system

The Rh blood group system classifies blood as Rh-positive or Rh-negative, based on the presence or absence of Rh antibodies in the blood.

often called the Rh factor, on the cell membranes of the red blood cells (erythrocytes). The designation Rh is derived from the use of the blood of rhesus monkeys in the basic test for determining the presence of the Rh antigen in human blood. The Rh blood group system was discovered in 1940 by Karl Landsteiner and A.S. Weiner. Since that time a number of distinct Rh antigens have been identified, but the first and most common one, called RhD, causes the most severe immune reaction and is the primary determinant of the Rh trait. (https://www.merriam-webster.com/dictionary/designation, seen 2016).

The Rh antigen poses a danger for the Rh-negative person, who lacks the antigen, if Rh-positive blood is given in transfusion. Adverse effects may not occur the first time Rh-incompatible blood is given, but the immune system responds to the foreign Rh antigen by producing anti-Rh antibodies. If Rh-positive blood is given again after the antibodies form, they will attack the foreign red blood cells, causing them to clump together, or agglutinate. The resulting hemolysis, or destruction of the red blood cells, causes serious illness and sometimes death. (Encyclopædia Britannica , seen 2016)

A similar hazard exists during pregnancy for the Rh-positive offspring of Rh-incompatible parents, when the mother is Rh-negative and the father is Rh-positive. The first child of such parents is usually in no danger unless the mother has acquired anti-Rh antibodies by virtue of incompatible blood transfusion. During labour, however, a small amount of the fetus's blood may enter the mother's bloodstream. The mother will then produce anti-Rh antibodies, which will attack any Rh-incompatible fetus in subsequent pregnancies. This process produces erythroblastosis fetalis, or hemolytic disease of the newborn, which can be fatal to the fetus or to the infant shortly after birth. Treatment of erythroblastosis fetalis usually entails one or more exchange transfusions. The disease can be avoided by vaccinating the mother with Rh immunoglobulin after delivery of her firstborn if there is Rh-incompatibility. The Rh vaccine destroys any fetal blood cells before the mother's immune system can develop antibodies.

Although the Rh-negative trait is rare in most parts of the world, it occurs in about 15 percent of Caucasians in Europe, Canada, and the United States. The trait's highest incidence is among the Basques of the Pyrenees (25–35 percent) and the Imazighen (Berbers) of Africa and

the Bedouins of the Sinai Peninsula (18–30 percent). (Encyclopædia Britannica , seen 2016).

1.2.1 .3 Minor blood systems

There are over 100 other blood subtypes. Most have little or no effect on blood transfusions, but a few of them may be the main causes of mild transfusion reactions. Mild transfusion reactions are frightening, but they are rarely life-threatening when treated quickly (Dean, Laura 2005).

The frequency of ABO blood group antigens varies in different populations. Most people have the antigen "H" encoded, because this is the precursor for antigen A or B. Thus, depending on whether the other genes are encoded, it will be determined if they will remain a type O or change to type A, B, or AB. Type O blood is the most frequent, and type B and AB are the least frequent (see Table 1-1).

Table 1-1. ABO Phenotype Frequencies Among Different Ethnic Groups (Adapted From Several Sources) (Victoria K Gonsorcik 2013)

Race	O	A	B	AB
White	%44	43%	%9	%4
Black	%49	%27	%20	%4
Asian	43%	27%	25%	5%

Asian 43% 27% 25% 5%In rare cases, even the initial precursor H antigen is not genetically encoded. These individuals are known as Bombay and are able to receive only Bombay type blood, because they make antibodies to not only type A, B, or AB donor RBCs but also to

type O donor red cells (anti-H), causing hemolysis of the transfused donor RBCs. The ABO system is regarded as the most important blood-group system in transfusion medicine because of severe hemolytictransfusion reactions and, to a lesser degree,hemolytic disease of the newborn.

1.2.1 .4 Other blood grouping systems

- The Duffy blood group

Duffy blood group system, classification of human blood based on the presence of glycoproteins known as Fy antigens on the surface of red blood cells, endothelial cells (cells lining the inner surface of blood vessels), and epithelial cells in the alveoli of the lungs and in the collecting tubules of the kidneys. (Kara Rogers 2002).

The Duffy glycoprotein is a receptor for chemicals that are secreted by blood cells during inflammation. It also happens to be a receptor for Plasmodium vivax, a parasite that invades red blood cells (RBCs) and causes malaria. RBCs that lack the Duffy antigens are relatively resistant to invasion by P. vivax. This has influenced the variation in Duffy blood types seen in populations where malaria is common. (Reid M.E and Lomas-Francis, 2003).

The Duffy antigens Fya (Fy1) and Fyb (Fy2) were discovered in 1950 and 1951, respectively. Antigens are named after the patient in whom Fya antibodies were first detected. Its act as receptors for substances called chemokines, which are hormone like molecules that attract cells of the immune system to particular sites in the body. The Duffy antigens also serve as receptors for the malarial parasites *Plasmodium knowlesi* and *P. vivax*. There are four possible Fy phenotypes: Fya+b+, Fya+b−, Fya−b+, and Fya−b−. Because Duffy antigens are not expressed in the Fya−b−phenotype—and hence there are no receptors to which

malarial parasites can bind—the null condition is associated with some degree of protection against malaria. Research has indicated that the increased frequency of the Fya−b−phenotype in West Africans and African Americans is the result of natural selection for disease resistance. (Kara Rogers 2002).

Antibodies formed against the Duffy antigens are a cause of both transfusion reactions and hemolytic disease of the newborn. (Reid M.E and Lomas-Francis 2003).

- MNSs blood group system

Classification of human blood based on the presence of various substances known as M, N, S, and s antigens on the surfaces of red blood cells. This system, first discovered in 1927, has many distinct phenotypes and is of interest in genetic and anthropological studies of human populations. (Daniels G. , 2002).

- Kell blood group system

Classification of human blood based on the presence on the surfaces of red blood cells of various antigens encoded by the *KEL* gene. The system, discovered in 1946, is characterized by a high degree of polymorphism(genetic variation), and thus studies of the Kell antigens have provided insight into the development of polymorphic traits in the context of human evolution. (Encyclopædia Britannica, seen at 2016)

1.2.1 .5 ABO grouping test

ABO grouping is a test performed to determine an individual's blood type. It is based on the premise that individuals have antigens on their red blood cells (RBCs) that correspond to the 4 main blood groups: A, B, O, or AB. Individuals have antibodies (isohemagglutinins) in their plasma that are directed against blood group antigens that their RBCs lack. These

antibodies (isohemagglutinins) form early in life. ABO antigens are expressed on RBCs, platelets, and endothelial cells and are present in body fluids. ABO testing is performed in order to prevent an adverse transfusion reaction that could be caused by ABO incompatibility between the patient and a blood donor. (Victoria K Gonsorcik, 2013).

Table 1-2. Genotyping of ABO

Blood Group	Antigens Present on RBCs	Antibody Present in Serum	Genotype
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen B antigen	None	AB
O	None	Anti-A, anti-B, anti-A,B	OO

Source: Victoria K Gonsorcik, (2013).

The gene *FUT1*, located on chromosome 19q13.3, is responsible for the synthesis of AB and H antigens. Chromosome 9q34 encodes for A and/or B glycosyltransferases. These are the different transferases necessary to produce the various ABO antigens (mainly glycolipoproteins) on blood components. A separate "secretor"/"se" gene (*FUT2*) is also located on chromosome 19q13.3; this gene encodes for the transferases necessary to produce ABO antigens (mainly glycoproteins) that are affiliated with bodily fluids other than blood (eg, saliva). Most of the population (approximately 80%) expresses the secretor gene. The precursor glycoprotein/glycolipoprotein that allows expression of all ABO antigens is composed of oligosaccharide chains with the essential addition of fructose. Alpha-2-L-fucosyltransferase is the enzyme responsible for adding fructose to the primary galactose of the oligosaccharide chain.

This is the foundation "H" antigen. The gene for type "O" is silent and thus maintains the original formation of the H antigen.

For the configuration of A, B, or AB antigens, an additional alpha 1,3-N acetylgalactosaminyltransferase and/or glycosyltransferase enzyme is encoded to attach additional sugars to the "H" antigen. For type A, an N-acetyl-galactosamine is attached to the primary, initial galactose. For type B, an additional galactose is attached to the primary galactose. For type AB, both of these sugars would be added. The O allele is an autosomal-recessive trait, and the A and B alleles are codominant traits. Each parent contributes an A, B, or O allele to their offspring, depending on the ABO type of the parents . (Victoria K Gonsorcik, 2013).

The ABO test may be part of a group of tests performed in various clinical settings. A type and screen includes ABO, Rh, and antibody detection/identification. ABO, Rh, antibody detection/identification, and compatible matching with a donor unit are included in a type and cross. A type and cross needs to be completed before blood is issued to prevent ABO incompatibility. (Reid ME and Lomas-Francis C . 2004).

Recipient organ/hematopoietic stem cell testing

A type and screen of the recipient is performed to assess ABO compatibility between the recipient and potential organ and hematopoietic stem cell donors. In hematopoietic stem cell transplantation, progenitor cells engraft into the recipient's chemotherapy-induced "empty" marrow and replace the recipient's blood ABO type and Rh type with that of the donor's if the engraftment is successful. (Stern *et al*, 2006).

Blood donor testing

A type and screen of the donor is completed as one of the required tests for blood donation. This allows blood-collection facilities to quickly assess the inventory status of ABO/Rh type by label identification. Before a donor unit is transfused, additional compatibility testing with the intended recipient's plasma/serum is competed. This will also act as a second validation step of the initial donor ABO/Rh typing. (http://www.redcrossblood.org)

Donor organ/hematopoietic stem cell testing

A type and screen of the donor is completed as one of the required tests for organ or hematopoietic stem cell donation. This is to assess the level of compatibility of the donor's and intended recipient's ABO/Rh types, which will dictate the subsequent type of blood components transfused. **Evaluation of hemolytic disease of the fetus and newborn associated with ABO incompatibility.**

Hemolytic disease of the fetus and newborn occurs when a fetus inherits paternal RBC antigens that the maternal immune system does not recognize as her own. A small percentage of fetal blood may come into direct contact with maternal blood circulation through fetal maternal hemorrhage (e.g. amniocentesis, trauma, miscarriage/abortion, placental abnormalities). (Sabita *et al* 2011).

The ABO system of fetal RBC antigens are not as fully developed in utero and are lesser in number. Mild hemolysis may result if there is ABO incompatibility between the baby and mother, as the maternal immune system does not easily recognize the incompatible ABO antigen, thus potentially averting a more serious hemolytic reaction.

The scenario that is indicted more often for increased severity of hemolysis is when the mother is type O and the fetus has either type A, B, or AB. The anti-A or -B antibodies that are produced from type O mothers are mainly immunoglobulin (Ig) G. IgG antibodies are smaller and are able to easily pass through the placenta, causing more likely exposure of fetal RBCs to the mother's antibody in the right clinical setting. (Sabita *et al* 2011).

Table 1-3 ABO groups antigens

Front/Forward typing Reaction of red cells tested with reagent antibody		Back/reverse Typing of patients serum tested against reagent red cells		Corresponding ABO group of patient's red cells
Anti-A (Antibody A)	Anti-B(Antibody B)	A Red cells	B Red cells	
0	0	+	+	0
+	0	0	+	A
0	+	+	0	B
+	+	0	0	AB

https://en.wikipedia.org/wiki/ABO_blood_group_system

ABO subtypes: These subtypes are defined as a blood type that has most of the chemical characteristics of type A, B, or O but that has a slight variation in a portion of the structure that can be recognized through testing. These changes may occur through various mutations, such as frameshift mutations, amino acid substitution, single point mutations,

single missense mutations, and deletions. The amount of variation determines if it is clinically significant enough to cause hemolysis.

The 2 most common subtypes of A are A1 (approximately 80% of all type A individuals) and A2 (approximately 20% of all type A individuals) . Most commercial testing reagents use A1 RBC antigen. Although there will be discrepancies between the front and back typing between an A2 individual cells and A1 reagent cells, this usually does not cause clinically significant hemolysis. If there is a concern, a blood bank research laboratory can use the chemical *Dolichos biflorus* to confirm the A1 status of a patient. Only A1 will react with *D biflorus*. Similar subtypes of B occur, but they are much rarer. (https://optn.transplant.hrsa.gov).

Table 1-4 Subgroups of A Identification.

Group	A1	A2	A3	Ax
Reaction with anti-A	4+	4+	Mixed field	0
Reaction with anti-A,B	4+	4+	Mixed field	2+
Reaction with lectin A1	4+	0	0	0
Reaction with lectin-H	0-w	1-2+	2+	2-3+
Presence of anti-A1	No	Maybe	Maybe	Often in serum

Source : Bodil Arnbak **(2016)**

Although uncommon, another discrepancy in ABO testing can result from certain bacterial infections and malignancies that may cause an acquired "B" typing from an A type individual. The patient's underlying disorder can cause enzymatic deacetylation of group A antigen, thus forming a B-like antigen, "acquired" B phenomenon. Although this will cause some weak discrepancies in the laboratory, the patient will still receive type A blood products. (Bodil Arnbak 2006).

ABO blood group system is the most important blood type system (or blood group system) in human blood transfusion. Found on platelets, epithelium, and cells other than erythrocytes, AB antigens (as with other serotypes) can also cause an adverse immune response to organ transplantation. The associated anti-A and anti-B antibodies are usually IgM antibodies, which are produced in the first years of life by sensitization to environmental substances, such as food, bacteria, and viruses. ABO blood types are also present in some other animals, for example rodents and apes, such s chimpanzees, bonobos, and gorillas.

1.2.1 .6 Role of ABO antigens in transfusion medicine

For a blood donor and recipient to be ABO-compatible for a transfusion, the recipient must not have Anti-A or Anti-B antibodies that correspond to the A or B antigens on the surface of the donor's red blood cells (since the red blood cells are isolated from whole blood before transfusion, it is unimportant whether the donor blood has antibodies in its plasma). If the antibodies of the recipient's blood and the antigens on the donor's red blood cells do correspond, the donor blood is rejected. On rejection, the recipient may experience Acute hemolytic transfusion reaction (AHTR).

In addition to the ABO system, the Rh blood group system can affect transfusion compatibility. An individual is either positive or negative for

the Rh factor; this is denoted by a '+' or '−' after their ABO type. Those with Rh-positive blood can safely receive both Rh-positive and Rh-negative blood, but those with Rh-negative blood should only receive Rh-negative blood. Rh-negative blood is used in emergencies when there is no time to test a person's Rh type. Because of this, the AB+ blood type is referred to as the "universal recipient", as there are neither Anti-B or Anti-A antibodies in its plasma, and can receive both Rh-positive and Rh-negative blood. Similarly, the O− blood type is called the "universal donor"; since its red blood cells have no A or B antigens and are Rh-negative, no other blood type will reject it. (Reid ME and Lomas-Francis C . 2004).

1.2.1 .7 Disease risks of blood groups

Compared to O group individuals, non-O group (A, AB, and B) individuals have a 14% reduced risk of squamous cell carcinoma and 4% reduced risk of basal cell carcinoma. Conversely, type O blood is associated with a reduced risk of pancreatic cancer. The B antigen links with increased risk of ovarian cancer. Gastric cancer has reported to be more common in blood group A and least in group O. (Xie J, Qureshi 2010).

According to Glass, Holmgren, et al., those in the O blood group have an increased risk of infection with cholera, and those O-group individuals who are infected have more severe infections. The mechanisms behind this association with cholera are currently unclear in the literature. (Glass 2012).

ABO hemolytic disease of the newborn

ABO blood group incompatibilities between the mother and child does not usually cause hemolytic disease of the newborn(HDN) because

antibodies to the ABO blood groups are usually of the IgM type, which do not cross the placenta. However, in an O-type mother, IgG ABO antibodies are produced and the baby can potentially develop ABO hemolytic disease of the newborn. (Xie J, Qureshi 2010).

		Donors							
		O−	**A−**	**B−**	**AB−**	**O+**	**A+**	**B+**	**AB+**
Recipients	**O−**	✓							
	A−	✓	✓						
	B−	✓		✓					
	AB−	✓	✓	✓	✓				
	O+	✓				✓			
	A+	✓	✓			✓	✓		
	B+	✓		✓		✓		✓	
	AB+	✓	✓	✓	✓	✓	✓	✓	✓

source : Glass 2012

Fig 1-1 ABO and Rh blood type donation showing matches between donor and recipient types

1.2.1 .8 RBC and ABO:

ABO blood group system was inherited:

Of all the RBC polymorphisms the most well known and medically important is the ABO blood group (Johnson and Hopkinson 1992), which was discovered in 1900 and 1901 at the University of Vienna by Karl Landsteiner in the process of trying to learn why blood transfusions sometimes cause death and at other times save a patient. The fact that the ABO blood group system was inherited was suggested in 1910 by Epstein and Ottenberg, while the confirmation of the ABO system being genetically inherited was suggested by von Dungern and Hirszfeld who studied 72 families with 102 children and found that the inheritance of the A and B agglutinogens obeyed Mendel's laws (Klug et al 1997).

The ABO system occurs as a result of polymorphism of complex carbohydrate structures of glycoproteins and glycolipids expressed at the surface of erythrocytes or other cells, or present in secretions, as glycan units of mucin glycoproteins, and the blood types are inherited through genes on chromosome 9, and they do not change as a result of environmental influences during life. The ABO gene is autosomal and therefore each person has two copies of genes coding for their ABO blood group (one maternal and one paternal in origin) and it was observed that the A and B blood groups were dominant over the O blood group and it was also found that the A and B group genes were codominant. This meant that if a person inherited one A group gene and one B group gene their red cells would possess both the A and B blood group antigens and these alleles were termed A (which produced the A antigen), B (with produced the B antigen), and O (which was "nonfunctional" and produced no A or B antigen) (Mudad, Raja; Telen, Marilyn J. 1996).

1.2.2 Malaria

Malaria in humans is caused by a protozoon of the genus Plasmodium and the four subspecies, falciparum, vivax, malariae, and ovale. The species that causes the greatest illness and death in Africa is *P. falciparum*. The disease is transmitted by the bites of mosquitoes of the genus Anopheles, of which the Anopheles gambiae complex (the most efficient) is responsible for the transmission of disease in Africa. Fever is the main symptom of malaria. The most severe manifestations are cerebral malaria (mainly in children and persons without previous immunity), anemia (mainly in children and pregnant women), and kidney and other organ dysfunction (e.g., respiratory distress syndrome). (WHO 2017).

Persons repeatedly exposed to the disease acquire a considerable degree of clinical immunity, which is unstable and disappears after a year away from the endemic-disease environment. Immunity reappears after malarial bouts if the person returns to an endemic-disease zone. Most likely to die of malaria are persons without previous immunity, primarily children or persons from parts of the same country (e.g., high altitudes) where transmission is absent, or ersons from more industrialized countries where the disease does not exist.

Malaria is a leading cause of child deaths in Africa. According to the Centers for Disease Control and Prevention, malaria is the 5th cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis). Malaria is the 2nd leading cause of death from infectious diseases in Africa, after HIV/AIDS. (WHO 2016).

About 3.2 billion people – nearly half of the world's population – are at risk of malaria. In 2015, there were roughly 214 million malaria cases and

an estimated 438 000 malaria deaths. Increased prevention and control measures have led to a 60% reduction in malaria mortality rates globally since 2000. Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden. In 2015, the region was home to 89% of malaria cases and 91% of malaria deaths. (WHO 2016).

In the last decade, the prevalence of malaria has been escalating at an alarming rate, especially in Africa. An estimated 300 to 500 million cases each year cause 1.5 to 2.7 million deaths, more than 90% in children under 5 years of age in Africa.

Malaria has been estimated to cause 2.3% of global disease and 9% of disease in Africa; it ranks third among major infectious disease threats in Africa after pneumococcal acute respiratory infections (3.5%) and tuberculosis (TB) (2.8%). Cases in Africa account for approximately 90% of malaria cases in the world. Between 1994 and 1996, malaria epidemics in 14 countries of sub-Saharan Africa caused an unacceptably high number of deaths, many in areas previously free of the disease. (Thomas Nehinda 1998).

The global malaria eradication program of the 1950s and 1960s suffered serious setbacks in the early 1970s, and the disease was slowly increasing in areas of Asia and South America where the number of cases had been reduced to low levels. However, about 90% of all malaria deaths in the world today occur in the sub-Saharan Africa because the majority of infections in the region are caused by Plasmodium falciparum, the most dangerous of the four human malaria parasites, and also because the most effective malaria vector—the mosquito Anopheles gambiae—is the most widespread and the most difficult to control. (WHO Africa Malaria Report 2003).

Although infection with malaria parasites is common, only 1 to 2% of infections lead to severe life threatening disease characterized by a range of clinical features, including unrousable coma (cerebral malaria), severe anemia, metabolic acidosis, and multiorgan failure (Oaks *et al*. 1990).

A recent upsurge of malaria in endemic-disease areas with explosive epidemics in many parts of Africa is probably caused by many factors, including rapidly spreading resistance to antimalarial drugs, climatic changes, and population movements. In Africa, malaria is caused by Plasmodium falciparum and is transmitted by Anopheles gambiae complex. Control efforts have been piecemeal and not coordinated.

Strategies for control should have a solid research base both for developing antimalarial drugs and vaccines and for better understanding the pathogenesis, vector dynamics, epidemiology, and socioeconomic aspects of the disease. An international collaborative approach is needed to build appropriate research in a national context and to effectively translate research results into practical applications in the field. The Multilateral Initiative for Malaria in Africa can combine all of the above strategies to plan and coordinate partnerships, networking, and innovative approaches between African scientists and their Northern partners.(Thomas 1998).

Important questions about control include the following. Is there enough knowledge about the disease and its determinants? Are there enough tools? Are existing resources adequate? Are governments and populations of endemic-disease countries adequately prepared?

The present strategy for malaria control, adopted by the Ministerial Conference on Malaria in Amsterdam in 1992, is to prevent death, reduce illness, and decrease social and economic loss due to the disease . polymerase chain reaction techniques should be explored. Also,

management guidelines should be developed concerning when and under what conditions to change the treatment regimen for different levels of resistance at the district, regional, and central level. The most promising ones, artemisinin and its derivatives artemether, arteether, and artesunate, are being tested for use in cerebral malaria and cases of proven resistance to chloroquine; some are already used in some countries.

The old vector-control method of house spraying persists in some countries. The relative merits and cost-effectiveness of house spraying versus the use of treated nets should be evaluated. Falciparum malaria is a complex disease with a patchy nonuniform distribution and clinical manifestations that vary from one area to another within an endemic-disease zone, often showing space-time clustering of severe malaria in the community (Snow RW, Marsh K. 2002).

The relationship between fevers, clinical disease, anemia, and cerebral malaria remains the subject of current research. The determinants of severe life-threatening malaria need further elucidation. Present research, focusing on the disease rather than the infection and the dynamics of its transmission, is bringing in new vision about the disease, particularly the immunologic aspects. Persons with asymptomatic parasitemia constitute an important reservoir. The epidemiology of malaria (particularly the relationship between the clinical patterns of the disease in different locations, the pattern of severe disease, and causes of deaths due to malaria) needs future research (International Conference on Malaria in Africa 1997).

1.2.2.1 Relationship between malaria and the RBC:

The invasion of RBCs, which follows the preerythrocytic phase of the life cycle is the basic pathological process in malaria infection. The parasite

must engage receptors on RBC for binding and undergo apical reorientation, junction formation, and signaling (S Josefin 2013).

The parasite then induces a vacuole derived from the RBC plasma membrane and enters the vacuole by a moving junction . Three organelles on the invasive (apical) end of parasites (rhoptries, micronemes, and dense granules) define the phylum Apicomplexa and receptors for invasion of RBC by merozoites and for invasion of liver by sporozoites are found in micronemes, on the cell surface, and in rhoptries. This sequence of RBC invasion, which is well established for *P. falciparum* infection, is probably similar for the three other species of the malaria parasite (Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae) and therefore establishes the direct relationship between malaria and the RBC (Adams *et al.* 1990).

1.2.3 ABO and malaria

Many pathophysiological mechanisms have been postulated to explain host susceptibility for parasite colonization and clinical presentation of the disease. Some studies have suggested that host susceptibility could be linked to the ABO blood group and/or Rh type due to the presence of different erythrocyte receptor systems that act as ligands to facilitate parasite adhesion (Tewodros *et al* 2011).

Plasmodium falciparum malaria is a known cause of morbidity and mortality especially in children of Sub-Saharan Africa (WHO 2008). The clinical outcome of *falciparum* malaria in endemic areas is, among other factors, associated with erythrocyte polymorphisms including the ABO blood groups. (Miller *et al* 2002). Numerous associations have been reported between the ABO blood group system and some disease conditions such as skin cancer , schistosomiasis , onchocerciasis , and HIV infection . (Liliane *et al* 2015) .

There are also reports on association of ABO blood group with susceptibility, resistance, and severity of *P. falciparum* malaria infection . Individuals with blood group "A" have been found to be highly susceptible to falciparum malaria whereas blood group "O" is said to confer protection against complicated cases . (N. Gopal Raj 2015) .

Low parasitaemia and uncomplicated *P. falciparum* malaria cases among blood group "O" individuals have been observed . (Tewodros *et al* 2011).

These differences in susceptibility and severity of *P. falciparum* malaria infection among the "A," "B," "AB," and "O" blood groups have been attributed to rosetting of parasitized erythrocytes and cytoadherence . (J. Alexandra Rowe *et al* 2007).

Rosetting contributes to the pathogenesis of severe malaria by obstructing microvascular blood flow . Studies have shown that rosetting is reduced in blood group "O" erythrocytes compared with the non-O blood groups (A, B, and AB) in *P. falciparum* laboratory and field isolates. Rosettes may form in blood group "O" cells but these rosettes are smaller and unstable compared to rosettes formed in non-O blood groups. It is, thus, presumed that blood group "O" may be a protective factor against severe malaria . (J. Alexandra Rowe *et al* , 2007).

Some studies have also reported the absence of significant association between ABO blood groups and *P. falciparum* malaria, so that the relationship between ABO blood group and malaria has not been clearly defined. Many studies aimed to confirm the association, or otherwise, between blood groups and complicated *falciparum* malaria. (Uneke , 2006). It was suggested that the ABO system has evolved under a positive selection pressure in both humans and other primates (N Kermarrec *et al.* (1999). The implication is that certain ABO groups provide a selective vulnerability to individuals possessing a particular

ABO blood group. Attempts were made to determine the significance of particular ABO phenotypes to disease susceptibility such as P. *falciparum* malaria. Although the relationship between blood group and susceptibility to malaria was studied by several researchers, results were contradictory and unable to establish an unequivocal link between ABO blood groups and the incidence of malaria parasitemia, malaria antibody levels, or the rate of repeat attacks of malaria (N. Gopal Raj , 2015).

However, the virulence of *P. falciparum* was associated with the capacity of the infected RBC to adhere to endothelial cells and to uninfected RBCs, a process known as rosetting, which was linked to the occurrence of severe malaria, i.e., cerebral malaria and anemia (Rowe *et al*. 2007).

Table 1-5 Summary of studies that investigated the association between *P. falciparum* rosetting and ABO blood group

Authors and year of publication	Origin of P. falciparum strains/isolates used	Order of frequency of rosette formation with ABO blood group	Significant association between ABO blood group and resetting
Carlson and Wahlgren 1992	Uganda Thailand	A, AB, B, O	Yes
Udomsangpetch et al. 1993	The Gambia	A, B, AB, O	Yes
Rowe et al. 1995	Kenya	AB, A, B, O	Yes
Barragan et al. 2000	The Gambia Thailand	A, B, O	Yes

source: Deepa *et al* (2011) *:*ABO blood groups and malaria .

Table 1-6 Summary of the studies that observed no significant association between *P. falciparum* malaria and ABO blood group

Authors and year of publication	Type of study	Study location	Main study populations	Malaria factor associated with ABO blood group	Statistical significant association
Martin et al. 1979	Cross-sectional	Nigeria	Children with severe malaria	Parasitemia	No
Kassim and Ejezie 1982	Cross-sectional	Southwestern Nigeria	Apparently healthy adults and children	Parasitemia	No
Bayoumi et al. 1986	Case-control	Central Sudan	Apparently healthy adults	Prevalence of malaria	No
Akinboye and Ogunrinade 1987	Cross-sectional	Ibadan, Nigeria	Apparently healthy adult blood donors	Parasitemia/antibody titer	No
Thakur and Verma 1992	Cross-sectional	Muria Gond and Delhi, India	Apparently healthy adults and children	Parasitemia/antibody titer	No
Montoya et al. 1994	Cross-sectional	Medellin, Colombia	Apparently healthy adults	Prevalence of malaria	No
Uneke et al. 2006	Cross-sectional	Southeastern Nigeria	Apparently healthy adult blood donors	Prevalence of malaria	No

source: Deepa *et al* **(2011)***:* ABO blood groups and malaria .

Table 1-7 Summary of studies that observed a significant association between *P. falciparum* malaria and ABO blood group

Authors and year of publication	Type of study	Study location	Main study populations	Malaria factor associated with ABO blood group	Statistical significant association
Pant et al. 1992	Cross-sectional	Kheda District, India	Apparently healthy adults and children	Prevalence of malaria	Yes
Singh et al. 1995	Cross-sectional	Madhya Pradesh, India	Adults and children with fever	Parasitemia	Yes
Fischer and Boone 1998	Cross-sectional	Kadoma, Zimbabwe	Adults and children with symptomatic malaria	Parasitemia/ hemoglobin levels	Yes
Lell et al. 1999	Case-control	Gabon	Children with severe malaria	Parasitemia	Yes
Migot-Nabias et al. 2000	Longitudinal/ cohort investigation	Gabon	Apparently healthy children	Parasitemia	Yes
Beiguelman et al. 2003	Cross-sectional	Portachuelo, Brazil	Apparently healthy adults and children	Number of malaria episodes	Yes
Pathirana et al. 2005	Case-control	Colombo, Sri Lanka	Adults and children with severe malaria	Parasitemia	Yes

source: Deepa *et al* **(2011):** ABO blood groups and malaria .

1.2 Previous studies:

1- The study of C. J. Uneke, 2006:

titled: *Plasmodium falciparum* malaria and ABO blood group: is there any relationship?

In this report, a systematic review and evaluation of scientific information and findings from studies investigating the relationship between *P. falciparum* malaria and ABO blood group are presented. The implications of findings from these studies and their association with the risk, management and treatment of severe malaria, public health policy, and operational research needs in the malaria endemic areas of the tropical and subtropical regions of the world are discussed.

And previous studies have implicated the ABO blood group type in rosetting (Rowe *et al*. 1995) and strain-specific blood group preferences for rosette formation were described previously (Carlson and Wahlgren 1992).

Eighteen studies, which provided sufficient information to enable meaningful and reasonable comparisons, were identified and used for this review. Seven studies reported no significant association between *P. falciparum* malaria and the ABO blood group (Uneke *et al.* 2006).

Findings from another seven studies clearly indicated the existence of a significant association between *P. falciparum* malaria and the ABO blood group (Pathirana *et al*. 2005). In the remaining four studies reviewed, the erythrocyte resetting in *P. falciparum* malaria was investigated and rosetting was established as a *P. falciparum* virulence factor, the expression of which is modified by ABO blood group (Barragan *et al.* 2000).

Absence of significant association between *P. falciparum* malaria and ABO blood group Six of the studies that reported no significant association between *P. falciparum* malaria and the ABO blood group were cross-sectional studies while one was a case-control study. All the seven studies were conducted in malaria endemic areas.

Four studies done in Nigeria and the other three studies were from India, Columbia, and **Sudan** . Factors investigated by the various authors, in association with ABO blood group, included malaria prevalence (Uneke *et al.* 2006), parasitemia (Kassim 2005), and parasitemia/parasite antibody titer (Thakur and Verma 1992). Bayoumi *et al.* (1986) examined adults claiming resistance to malaria who were identified in **the** Sennar region of central Sudan**,** where *P. falciparum* is hyperendemic but seasonal in transmission and found no association with ABO blood groups. In the Columbian study, although malaria infection by P. falciparum was found in 91.4% of malaria-infected blacks, Montoya et al. (1994) observed no significant differences between the presence of malaria infection and ABO antigens.

2- The study of Hailu Tadesse 2011:

titled: Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia.

Background & objectives: There is lack of adequate information on the association between severe malaria and some human genetic markers like ABO blood types. The study was undertaken to evaluate the association between severe malaria infection and ABO blood types among febrile patients attending Felegeselam Health Center, northwestern Ethiopia.

Methods: A total of 398 febrile patients were examined for malaria and tested for ABO blood groups in December 2011. The blood samples were

collected by finger pricking, stained with Giemsa and slides were examined microscopically. ABO blood group was determined by agglutination test using agglutinating A and B monoclonal

anti-sera together with parasite load count. Chi-square and ANOVA tests were used to assess the difference between frequencies and means, respectively.

Results: Out of 398 acute febrile patients, 201 (50.5%) were found to be infected with *Plasmodium* parasites. Of which 194 (48.74%) and 7 (1.76%) belong to *Plasmodium falciparum* and *P. vivax*, respectively. The distribution of ABO blood groups was O (46%), A (27.1%), B (23.1%) and AB (3.8%). The percentage of severe malaria with respect to blood group A, B, AB and O was found to be 40, 34.1, 14.3 and 5.1%, respectively. The association of severe malaria with non 'O' blood types was statistically significant ($\chi 2 = 31.246$, $p < 0.01$). *Interpretation & conclusion:* The present findings indicate that individuals with blood groups A, B and AB are more susceptible for severe malaria infection than blood group O.

3- The study of Sara Muawia 2017 :

titled: Association of ABO Blood Group and Malaria Parasitic Infection in Eiedbabkier, Sudan.

Background: Malaria is an infection caused by protozoan parasites of the genus *Plasmodium*, and transmitted by the bite of infected Anopheles mosquitoes. Many studies reported an association between malaria and parasitic density with a different ABO blood group, but with contradictory results.

Objective: The purpose of this study was to determine the association between ABO blood groups and Malaria parasitic infection.

Methods: Two hundred and six samples from Sudanese population were included in this study (103 of them were Malaria patients and the other 103 were healthy controls), all of them were evaluated to determine the ABO blood groups. The blood group of the study participants was determined by direct ABO method.

Results: in our study there was a significant association between the ABO blood group and malaria infection with (p value 0.013). The most frequent blood group among patient group was A (42, 40.8%), followed by O (28, 27.2%), B (26, 25.2%), and at last AB (7, 6.8%).

Conclusion: The current study revealed that most frequent blood group in patient group was A, followed by O, B, and eventually AB, the males were more affected by malaria than females and blood group B is more susceptible to the infection by malaria than other blood groups.

1.3 Objectives :

General objective :

To determine the association between ABO blood group and Malaria infection.

Specific objectives:

- To evaluate malaria in some endemic-disease areas, with relation to the blood grouping.

- To Correlate between distribution of blood grouping with Malaria infections of Sudanese.

- To Correlate between distribution of blood grouping with ages and gender of Sudanese infected with Malaria.

Chapter two

Materials and methods

2.1 Study design and duration

A cross-sectional study of patients who diagnosed as malaric in the endemic Aljazeera- Central Sudan *"24-Algurashi"* rural hospital , was performed during April 2017. Personal data of patients were reported, the blood group ABO and Rh classification were included.

2.2 Study area and population

location: Aljazeera state Health centers, Central Sudan.

The study population was composed of outpatients who are seeking medical assistance at this hospital.

2.3 Inclusion criteria

Patients with Malaria positive test result were included, while those of negative result were excluded.

2.4 Ethical approval

The study obtained ethical clearance from the Sudan University of science and technology, and from the hospital administration, and verbal agreement from patients.

2.5 Clinical and laboratory diagnosis

Before collecting blood sample, explanation about the study was given and a written informed consent was obtained from every study participant including the guardians of children.

2.6 Sample collection

Capillary blood was collected by finger pricking using 70% isopropanol and sterile disposable lancet. Heel puncture was used for infants. Immediately, thin film was spread on grease free, frosted end, labeled slide using a smooth edged slide spreader. Thick film was also prepared on the same slide. Thin film was then fixed with methanol. (WHO publications 2005).

2.7 Staining of blood film

patients were examined in detail and screened for *P. falciparum* infection by Giemsa -stained thick and thin blood smears (SD Bio Standard Diagnostics India).

The blood film was stained with 10% Giemsa for 10 minutes. Finally, the films were examined under an oil immersion microscope objective (100x). Parasitemia was estimated for febrile patients who tested positive for P. falciparum by counting the number of parasites (asexual forms only) against 200 white blood cells (WBC). This counting was done by using hand tally counters. Then, the number of parasites per microliter of blood was calculated (WHO publications 2005).

Similarly, the blood group of the study participants was determined by direct slide method, using agglutinating A and B Monoclonal ERYCLONE® anti-sera alongside with the former procedures.

2.8 ABO blood group typing

Blood samples of falciparum-infected individuals were typed by commercial haem agglutination kit (Tulip Diagnostics, Goa, India). In brief, about 20 ul of whole blood was taken on a clean slide and 20 ul of antisera A, B and Rhesus blood group applied, mixed by means of an applicator stick and results were noted.

ABO blood groups were typed by the agglutination method using commercial antisera (Span Diagnostics Ltd., India).

If there is agglutination, this will indicate a positive reaction. The amount of agglutination is graded on a scale of 0 to 4.

2.9 Data analysis

Data were entered in Microsoft Excel, checked for its correctness, then by SPSS package every variable had been correlated to the rest of variables in different Excel sheets. simple statistic summations and percentages were done manually. Chi-square test was used to assess the difference between frequencies (the associations between blood groups and *P. falciparum* malaria cases).

Chapter Three

Results

A test group of 150 persons had been chosen randomly from the outpatients referred for laboratory investigations for Malaria, at *"24-Algorashi"* Hospital in Aljazeera State, central Sudan. With no specific criteria other than the time limits of the study which was April 2017. This 150 patients represents the general group in this research, while the patients who showed a positive malaria test represents "the infected group".

3.1 Characteristics of the general group (n=150)

Table 3-1 Sex distribution of the general group (n=150)

sex	No.	%
female	74	49.33
male	76	50.67
total	150	100%

Table 3- 2 Age distribution of the general group (n=150)

Age group (years)	No.	%
Less than 10	49	32. 67
10-20	18	12.00
21-30	26	17. 33
31-40	29	19. 33
More than 40	28	18. 67
total	150	100%

The general group were consisted of 74 female and 76 males, distributed to different age groups, 49 patients were Less than 10 years old, 18 patients 10-20 years , 26 patients 21-30 years , 29 patients 31-40 years , and 28 patients more than 40 years old.

Table 3- 3 BF for malaria of the general group (n=150)

BF for malaria	No.	%
positive BF	114	76
negative BF	36	24
total	**150**	**100%**

After laboratory investigations for Malaria, using thick blood films, 114 smears out of the 150 group found positive (parasite detected) (76% were positive of Malaria parasite), while 36 smears were negative (no parasite detected) (24%). This positive smears (114 patients) will be considered as our working group and to be called (infected group in this study).

Table 3- 4 ABO distribution of the general group (n=150)

ABO	No.	%
O+	79	52.67
O-	5	3.33
A+	41	27.33
B+	19	12.67
A-	3	02.00
AB +	3	02.00
total	150	100%

The blood group O+ is dominant in our general group (52.67%). Next prevalent is the A+ group (27.33%), then the B+ group (12.67%). The blood groups O- , A-, and AB+ are few (3.33% , 3% and 3% respectively)

3.2 Readings of the <u>infected</u> group (n=114)

Table 3-5 Gender of infected group (n=114)

gender	**No.**	**%**
females	53	46.49
male	61	53.51
total	**114**	**100%**

Table 3-6 Gender and age distribution of the infected group (n=114)

age group	Males (out of 61)	Females (out of 53)	Total (both sex)
under 10 years	**26** (42.52%)	**22** (41.51%)	**48** (42.11%)
10-20y	**8** (13.11%)	**7** (13.21 %)	**15** (13.16%)
20-30	**5** (8.20%)	**9** (16.98 %)	**14** (12.28%)
31-40	**8** (13.11%)	**8** (15.09%)	**16** (14.06%)
over40	**14** (22.95 %)	**7** (13.21 %)	**21** (18.42%)
total	**61** (100%)	**53** (100%)	**114** (100%)

Most of the 61 infected males are children (under 10 years) (42.52%). While (22.95 %) of infected males are aged over 40 years. Most of the 53 infected females are children (under 10 years) (41.51%). While other age groups of infected females are almost equally distributed . The majority of both sex of the infected group are children (under 10 years) (42.11%).

Table 3-7 ABO distribution of the infected group (n= 114)

ABO	No. of patients	%
O+	64	56.14
O-	2	1.75
A+	28	24.56
A-	3	2.63
B+	14	12.28
AB +	3	2.63
total	**114**	**100%**

Table 3-8 ABO distribution of the infected males (n= 61)

ABO	No. of males	%
O+	36	59.02
O-	2	3.28
A+	15	24.59
A-	2	3.28
B+	5	8.20
AB +	1	1.64
total	**61**	**100%**

Table 3-9 ABO distribution of the infected females (n= 53)

ABO	No. of females	%
O+	28	52.83
O-	-	0
A+	13	24.53
A-	1	1.89
B+	9	16.98
AB +	2	3.77
total	**53**	**100%**

Among 114 patients (the patients of positive malaria test), 28 were 'A' positive, 14 'B' positive, 64 were 'O' positive and 3 were 'AB' positive.

Table 3-10 Distribution of the blood groups between ages of the infected group.

age / ABO	O+	O-	A+	A-	B+	AB+
under 10 years	**28 /48** = (58.33%)	**0/48** = (0%)	**17/ 48** = (35.42%)	**1/ 48** = (2.08%)	**2/ 48** = (4.17%)	**0/ 48** = (0%)
10-20y	**7/15** = (46.67%)	**0/15** = (0%)	**5/15** = (33.33%)	**1/15** = (6.67%)	**2/15** = (13.33%)	**0/15** = (0%)
21-30	**8/14** = (64.29%)	**1/14** = (0 %)	**0/14** = (0%)	**0/14** = (0%)	**5/14** = (35.71%)	**0/14** = (0%)
31-40	**10/16** = (62.50%)	**1/16** = (6.25%)	**1/16** = (6.25%)	**0/16** = (0%)	**2/16** = (12.50%)	**2/16** = (12.50%)
over40	**11/21** = (52.38%)	**0/21** = (0 %)	**5/21** = (23.81%)	**1/21** = (4.76%)	**3/21** = (14.29%)	**1/21** = (4.76%)
total	**64/114 (57.02%)**	**2/114 (0%)**	**27/114 (23.68%**	**3/114 (2.63%)**	**14/114 (12.28%)**	**3/114 (2.63%)**

Out of 48 infected children (under 10 years old) , 28 child are of O+ blood group. i.e (58.33%) of the children (under 10 years old) had blood group type O+ . While (53.42%) of these children are of A+ blood group.

The prevalence of A- blood group and B+ blood group is very low in these infected children (2.08%) (4.17%) respectively. None of the infected children are of O- or AB+ blood groups.

More than half of the number of the age group (10-20 y) of the infected people, had O+ blood group (64.29%). While (33.33%) of this age group are A+. Few of these young people are of A- and B+ blood groups : (6.67%) and (13.33%) respectively. None of this age (10-20y) classified as O- blood group or AB+ blood group.

Out of 14 infected patients (21-30 years old) , 8 patients are of O+ blood group. i.e (64.29%) of the patients (21-30 years old) had blood group type O+ . The B+ grouped are (35.71%). While none of these patients had A+ blood group.

Out of 16 infected patients (31-40 years old) , 10 patients are of O+ blood group. i.e (62.50%) of the patients (31-40 years old) had blood group type O+ . The other blood groups are few : A+ , B+, AB+ : (6.25%) (12.50%) (12.50%) respectively. None of this age patients had O- blood group.

Out of 21 infected patients (over40 years old) , 11 patients are of O+ blood group. i.e (52.38%) of the patients (over40 years old) had blood group type O+ . While (23.81%) of these patients are of A+ blood group. Few patients had A- , B+ , AB+: (4.76%) (14.29%) (4.76%) respectively. None of this age group had O- blood group.

3.3 Correlations by SPSS

Table 3-11 correlation of Blood groups and Malaria infection

BG * BFFM Cross tabulation

BG	positive	negative	
O+ve	64	15	79
A+ve	28	13	41
B+ve	14	5	19
AB+ve	3	0	3
O-ve	2	3	5
A-ve	3	0	3
Total	114	36	150

Table 3-12 correlation of Blood groups and Malaria infection (Chi-Squire Test)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.927[a]	5	.160
Likelihood Ratio	8.678	5	.123
Linear-by-Linear Association	.825	1	.364
N of Valid Cases	150		

a. 7 cells (58.3%) have expected count less than 5. The minimum expected count is .72.

Table 3-13 correlation of Blood groups and Malaria infection (Symmetric Measures)

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.074	.082	.908	.365[c]
Ordinal by Ordinal	Spearman Correlation	.106	.081	1.291	.199[c]
	N of Valid Cases	150			

a. Not assuming the null hypothes

b. Using the asymptotic standard error assuming the null hypothes

c. Based on normal approximation

Chapter four

Discussion and Conclusion

4.1 Discussion

4.1.1 Patients ages

Malaria affected all age groups ranged from less than 10 up to more than 40 years old (M : F 6 : 5). This is similar to the study of J Vector Borne (2011), which indicated that Malaria affected all age groups and the age ranged from less than 10 up to more than 40 years old (M : F 8 : 2). The difference between infected age groups was not significant (P>0.05).

But this study is different from our study in the ratio of infected children compared to adults, in our research the number of adults affected (n= 52) was nearly equal to the number of children (n = 48), while that study had reported that the number of adults affected was more (n = 82) than the children (n = 18).

4.1.2 ABO blood groups and malaria infection

- When the Malaria infection was compared with blood groups, it was observed that 'O' group had an advantage (table 3-7).

- Individuals with blood group O+ (56.14%), were more infected than those of group A+ (24.56%), the difference between infected age groups was not significant (p >0.05).

- Among 114 patients (the patients of positive malaria test), 28 were 'A' positive, 14 'B' positive, 64 were 'O' positive and 3 were 'AB' positive.

- Our result about association between malaria and blood groups is similar to the result reported by J Vector Borne (2013), which found that

among 100 patients, 22 were ‘A’ positive, 42 ‘B’ positive, 35 ‘O’ positive and 1 ‘AB’ positive..

But it is not correspondent to the results of the study of Sara Muawia (2017) which shoewd a significant association between the ABO blood group and malaria infection with (p value 0.013). The most frequent blood group among patient group was A (42, 40.8%), followed by O (28, 27.2%), B (26, 25.2%), and at last AB (7, 6.8%).

- In some international studies bivariated analysis to establish association between severity of the disease and the ABO and Rh blood group classification did not show statistical significance for any of the blood types (C. J. Uneke, 2006). Those studies showed the distribution of ABO phenotypes among patients was O (52.83%), A (24.53%), B (16.98%) and AB (3.77%). No patients with blood group O was Rh (–)ve.

This result is in accordance with some international studies which had shown a high percentage of O blood group (51.3%) phenotype was observed among the study participants followed by A (23.5%), B (21.6%), and AB (3.3%). This agrees with some previous studies that also reported high frequency of group ‘O’ and low frequency of group ‘A’ phenotypes in tropical regions where malaria is rampant and widespread (Uneke 2006).

- Our results disagree with some other similar studies such as the study of (Tewodros 2011) which reported that in general, malaria infection showed significant association with blood group (P=0.015) with the highest proportion (77.8%) observed among individuals with AB blood group, followed by those with blood group B (64.4%).

- In the present study, high proportion of blood group O (52.83%) phenotype was observed among the study participants. This agrees with

some previous reports (51.3%) in southern Ethiopia, (55.83%) in Amazon region of Brazil and 54.4% in Zimbabwe which showed high frequency of group 'O' than non 'O' phenotypes in tropical regions where malaria is prevalent. (Uneke C.J. 2007).

- In the Indian scenario, the literature relating to malaria and the blood groups are sparse and have mixed results. Thakur and Verma (1992) in their study, concluded that ABO blood groups do not show differential susceptibility to malaria. Miller et al Joshi et al reported no correlation between ABO blood groups and malaria in Delhi. Other studies indicated a possible relationship.

4.1.3 ABO blood type and P. falciparum parasitaemia

Our findings show that most of the smears infected with (one cross " + ") parasite density, i.e (98%)were of low parasitaemia (not shown in our tables). Some international studies (Tekeste 2010**),** found a statistically significant difference of parasitaemia between different blood groups ($P<0.01$, $F= 11.510$).

4.2 Conclusion

1- O+ blood group is the most blood group among the population of Sudan.

2- No significant association between blood group and Malaria infection in Sudan.

3- Both sex and all ages groups are infected without significant association.

Recommendations

- Further studies are needed to set possibility of association between Malaria and blood group systems.

- No need to detect the blood group of Malaria patients, as no clear association was reported by this study.

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