بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and scientific Research

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Frequency of ABO, A-Sub Group and Rhesus D Antigen Among Alrashida Tribe in Shendi Town

A thesis Submitted for partial fulfillment of the MSc .Degree in Haematology

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August – 2018

الآيـــة

قال تعالى:

﴿ لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ)

صدق الله العظيم ﴾.

(سورة البقرة : الآية (٢٨٦))

Dedications

TO …….

The candle which burns to light my life
My mother

That who gave me kindness and tenderness
My father

Those who have made it possible
My teachers
The one who helped me very much
My supervisor

To who encourage me

My sisters ,my brothers and my friends

And special dedication to

Dr: Elfatih Mohammed Abd allah

Acknowledgements

First I would like to thank God for every thing…
I would like to express my immense gratitude and appreciation to my supervisor **Dr: Hamza Ahmed Hassan**
for the helpfulness and suggestion.
Iam deeply indebted to the people of Alrashida tribe for participation in this study .
And finally all our thanks to every one who supported and helped me through the study and were not mentioned.

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List of abbreviations

Age :	Antigen
AHG:	Anti _Human Globulin
CMV:	Cyto Megalo Virous
EDTA:	Ethylene Di amine Tetra Acetic acid
HD FN:	Hemolytic Disease of Fetus and New born
IgG:	Immunoglobulin G
IgM :	Immunoglobulin M
IS:	Immediate Spin
RBCs:	Red Blood Cells
Rh:	Rhesus blood group system
RHIG:	Rh-Immuno Globulin

Abstract

This is a descriptive cross sectional study which took place in Sudan in southern Shendi town.

The study aimed to determine the frequency of –ABO and –Rh blood group antigens and phenotypes among Alrashida tribe .

This study was conducted during the period of six months between March and August 2018, following informed consent.

A total of fifty venous blood samples were collected from un related individuals into 2.5 ml EDTA containers, the red blood cells were tested for –ABO by tube agglutination techniques and Rh antigens by slide agglutination techniques ,the alleles and frequencies were determined ,result were analyzed and the similarities were detected.

The antigen-O was the most common Ag among the study group (52%) followed by –A1(28%) ,the least common was–B (20%) and -AB antigen represented (0%).

The –D antigens was the commonest alleles detected with frequencies of (100%).

Similarities between Alrashida and Sudanese population and differences with others were noticed .

ملخص البحث

هذه دراسة وصفية مقطعية أجريت في السودان في جنوب مدينة شندي، وهدفت إلى تحديد فصيلة الدم والمستضدات في قبيلة الرشايدة. RH والـ ABO

وقد أجريت هذه الدراسة خلال فترة ستة أشهر بين مارس وأغسطس ٢٠١٨م بعد الموافقة المسبقة.

جمعت مجموعة٥٠عينة من الدم الوريدي من أفراد قبيلة الرشايدة ليست بينهم صلة قرابة في وعاء سعته ٢,٥مل يحتوى على مانع تجلط EDTAا تم اختبار خلايا الدم الحمراء بطريقة تراص الأنبوب والعامل الريصى بطريقة تراص الشريحة ,ثم تم تحديد الزمر الوظيفية والشكل الظاهري لكل عينه وحللت النتائج وتم الكشف عن أوجه التشابه.

الزمرة الوظيفية (O) كانت هي الأكثر ترددا بنسبة (٥٢%)تليها الزمرة A1 بنسبة (٢٨%) بينما كانت الزمرة (B) بنسبة (٢٠%) إما الزمرة (AB) لم تمثل نسبة في مجموعة الدراسة .

وفى النظام الريصى كانت الزمرة (D) هي الأكثر ترددا بنسبة (١٠٠%).

نجد أن هناك تشابه واختلاف في الزمر الوظيفية لفصائل الدم والعامل الريصى بين قبيلة الرشايدة وبعض القبائل السودانية

Chapter one

Introduction

Rationale

Objectives

1. Introduction

1.1. The ABO Blood Groups:

The ABO system was the first blood group recognized and remains preeminent in blood transfusion practice.

In 1900, Landsteiner tested red cells and sera from his laboratory workers and noted that the sera from some workers agglutinated the cells of others but not their own.

He then divided individuals into three groups (A, B, and O) based on these experiments and in 1902, Von Decastello and Sturli found the fourth group, AB. The blood group classified into four main groups, AB, A, B and O which are determined by the presence or absence on the red cells of two antigens A and B. The frequency of the ABO groups differs in different geographical regions but in Yemen it is approximately; group O 49%; A30%; B18% and AB 3%.

Transfusion of ABO-incompatible blood results in an acute hemolytic reaction which be life threatening.

The antigens are under the control of three allelic genes, A, B and O situated on the long arm of chromosome 9 and are inherited in a simple Mendelian fashion[(1).]

The frequency of ABO groups varies in different ethnic populations and this must be taken into account when recruiting representative blood donor panels. For example, people of Asian origin have a higher frequency of group B than white Europeans. Individuals of blood group O are sometimes known as universal donors as their red cells have no A or B antigens.

However, their plasma does contain anti-A and anti-B that, if present in high titre, has the potential to haemolyse the red cells of certain non-group O recipients [(2)]

1.2.The Rh system:

There are five main Rh antigens on red cells for which individuals can be positive or negative: C/c, D and E/e. RhD is the most important in clinical practice.

Around 85% of white Northern Europeans are RhD positive, rising to virtually 100% of people of Chinese origin.

Antibodies to RhD (anti-D) are only present in RhD negative individuals who have been transfused with RhD positive red cells or in RhD negative women who have been pregnant with an RhD positive baby. IgG anti-D antibodies can cause acute or delayed haemolytic transfusion reactions when RhD positive red cells are transfused and may cause haemolytic disease of the fetus and newborn (HDFN).

It important to avoid exposing RhD negative girls and women of child- bearing potential to RhD positive red cell transfusions except in extreme emergencies when no other group is immediately available.(2)

The ABO and Rh blood groups are the most important blood group systems in humans with tremendous variability among different races and ethnic groups. Their clinical importance is evident in blood transfusion and hemolytic disease of the newborn. A number of associations have been reported between diseases and blood group systems.(3)

1.2. Rationale

In Sudan the migration routes were variable because Sudan inhabited by different populations that migrate from other parts of Africa and Arabia so the interaction is very likely, this resulted in population with different characteristics , and different gene complex would be expected .

There are well –defined differences in the incidence of blood group antigens between people of different ethnic origins.

This research will provide an essential data about Alrashida ethnic group for scientific and proper protocol to be used in blood transfusion practice , the collected information can be used as guide for other studies like the association between phenotype and diseases.

More ever blood groups have very important role in transfusion medicine and forensic medicine application in the detection of certain criminal clinical cases , also can be used in parents identification and in compatibility investigation before organ transplantation.

1. 3. Objectives

1.3.1 General Objective:

To determine the patterns of ABO, A-sub groups and – Rhesus D antigen in Alrashida tribe.

1.3.2. Specific Objectives:

1- To determine the possible -ABO phenotype and A-sub group frequencies among Alrashida tribe.

2- To determine the possible – Rhesus D antigen among Alrashida tribe.

3- To compare the –ABO and Rhesus D antigen frequencies with other Sudanese tribes.

Chapter Two

Literature Review

2. Literature Review

2.1. ABO System:

The ABO system is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals have antibodies in their serum to antigens that are absent from their RBCs. This occurs without any exposure to RBCs through transfusion or pregnancy.

Due to the presence of these antibodies, transfusion of an incompatible ABO type may result in immediate lysis of donor RBCs.

This produces a very severe, if not fatal, transfusion reaction in the patient. [4]

In 1901, Landsteiner drew blood from him self and five associates, separated the cells and serum, and then mixed each cell sample with each serum. He was inadvertently the first individual to perform forward and reverse grouping.

(Forward grouping) is defined as using known sources of commercial anti sera (anti-A, anti-B) to detect antigens on an individual's RBCs.

Reverse grouping is defined as detecting ABO antibodies in the patient's serum by using known reagent RBCs, namely A1 and B cells. [4]

2.1.1.Inheritance of ABO blood groups:

Bernstein first described the theory of inheritance of ABO blood groups in1924.

He demonstrated that each individual inherits one ABO gene from each parent and the presence of these two genes determines the type of antigen present on the surface of red cells. The gene A, B or O occupy one locus on each chromosome.[5]

2.1.2. ABH antigens:

Soluble –A-Band-H-antigens are glycol proteins and the difference in the terminal e determine the specificity of these antigens; L.fucose; L.fucose +N acetyl. D-glactoseamine for A and L.fucose +D glactoseamineo for –B fifteen amino acids make up the protein back bone of the soluble antigen and four sugar (the above, plus Nacetyl. D glactoseamine) from side has no bone.

Cellular –A-B and –H antigens are of glycoproteins and glycolibids these structures have the same terminal sugars as the soluble-A,-B and H-

glycoprotiens and the – A and –B are the same as –H but with an immunodominant sugars added ;-D –glactoseamine for-B and glactoseamine N acetyle for-A.hence ABO terminates are oligosaccharides that are synthesized by specific transferase that are the products of the –ABO genes [3].

2.1.3.H antigen:

Group O cells have no antigens of the ABO system but do possess H substace, the precursor upon which the products of the *ABO* genes act. The *H* gene is on chromosome 19,whereas *ABO* is on chromosome 9.

The H antigen is present to some extent on almost all red cells, regardless of the ABO group ,but the amount of H antigen varies with the ABO group as follows: O > A2 > A2B > B > A1 > A1B.

2.1.4.Distribution of the A, B and H antigens:

ABH antigens are often referred to as histo-blood group antigens because they are widely distributed in the body. They are therefore very important in transplantation.

They are present on white cells, platelets and epidermal and other tissue cells.

They are also present in the plasma, regardless of ABH secretor status, and in the saliva and other secretions of ABH secretors [3].

2.1.5. A -Subgroups:

In 1911, von Dungern described two different A antigens based on reactions between group A RBCs and anti-A and anti-A1. Group A RBCs that react with both anti-A and anti-A1 are classified as A1, whereas those that react with anti-A and not anti-A1 are classified as A2. RBCs from A1 and A2 individuals react The A subgroups are generally more common ,The weaker serologic reactivity of ABO subgroups is attributed to the decreased number of A and B antigen sites on their red cells.

Classification into A1 and A2 phenotypes accounts for 99% of all group A individuals. The cells of approximately 80% of all group A (or AB) individuals are A1 (or A1B), and the remaining 20% are A2 (or A2B) or weaker subgroups. The differences between A1 and A2 are both quantitative and qualitative [4].

2.1.6. Rare ABO variants:

Rare ABO variants are usually disclosed because an expected ABO antibody is missing, a sample typed as group O that has anti-B but no anti-A will usually prove to be a weak A variant, the presence of weak A or B antigens can be demonstrated either by using potent antisera or by adsorption and elution. Avariety of A and B variant phenotypes exist with the symbols A3,Ax, A end, Am, A el, B3, B x, B m and B el.

All are extremely rare, are usually recognized by their variable reactions with anti-A and/or anti-A,B sera, and arise from mutations in the coding or regulatory regions of the *ABO* gene.

Weakening of the A antigen can occur in acute myeloid leukaemia.

The A antigen may revert to almost normal in remission similar weakening of B, H and I has been described.B-like antigens may be acquired by group A individuals who are suffering from bowel infections, usually associated with carcinoma or strictures of the large bowel.

Red cells with an acquired B antigen are agglutinated by some anti-B, including some monoclonal anti-B but not by the patient's own anti-B. Bacterial deacetylases convert *N*-acetylgalactosamine, the immunodominant sugar of the A antigen, into galactosamine,a structure similar to galactose, the immunodominant sugar of the B antigen.[3]

2.1.7. Bombay blood group (Oh phenotype):

The O blood group individuals do not carry either A or B antigen, but have maximum amount of H antigen on their red cells. Some individuals lack even H antigen along with A and B. These individuals are called Oh phenotype.

Since there is no H antigen on the surface of red cells of Oh, the anti-H antibody develops in their serum, along with all the other antibodies found in any O blood group.

The anti-H present in Oh is clinically significant, warm antibody reactive at 37°C. Bhende YM, et al in year 1952, first discovered this blood group in the city of Bombay, India, from where it got its name.

The Bombay blood group is not compatible with any ABO blood group, and the choice of blood for these individuals remains only Bombay itself [5].

2.1.8. ABO antibodies:

ABO antibodies are generally IgM in nature. They are naturally occurring, complete and cold reacting antibodies, which do not cross placental barrier, and are capable of binding the complement.

If the antigen is missing in a blood group, the corresponding antibody is present.

The anti-AB of O blood group carries a higher titre than anti-A or anti-B.

The anti-A and anti-B present in O blood group are more often IgG in nature and are known as haemolysins.

2.1.9. Antibodies in infants:

The IgM anti-A and anti-B are not produced up to the age of 3 to 6 months.

The maximum titre reaches by the age of 5 to 10 years.

What ever antibodies are present in a newborn are of maternal origin.[4]

2.2. Rhesus Blood Group System:

2.2.1.General introduction to Rh system:

The Rh blood group system is a complex system, and certain aspects of its genetics and nomenclature are still unsettled.

The human antibody directed against the D antigen was first noticed in the serum of a group O woman who had a history of stillbirths and transfusion reactions. Levine and Stetson reported it in the year 1939.

In 1940, Landsteiner and Wiener raised an antibody from the serum of guinea pigs and rabbits by immunising them with the red cells of Rhesus monkey.

The same antibody agglutinated the red cells of 85% of the humanbeings.

The antibody was called anti-Rh, and its antigenic determinant Rh factor due to its similarities with the antigen found in the Rhesus (Rh) monkey.

Wiener and Peters in the year of 1940 isolated human anti-Rh antibody from the sera of individuals transfused with ABO compatible Rh positive blood. Further studies established that the animal anti-Rh and human anti-Rh are not identical,

but by that time it was too late and Rh blood group system had received its name[5]

2.2.2. Rh antigens:

Alloanti-D was initially considered to be identical to antibodies produced in rabbits and guinea pigs immunized with rhesus monkey red cells.

A remnant of the original name, rhesus, remains in the name of the blood group system, Rh. For clinical purposes, individuals are often called Rh-positive if they have the D antigen and Rh-negative if they lack D. Approximately 85% of Caucasians, 95% of Africans and over 99% of eastern Asians are D-positive.

Two pairs of antithetical antigens, C (RH2) and c (RH4), and E (RH3) and e (RH4), are closely associated with D genetically.

There is no antithetical antigen to D, so d represents the absence of D.

From serological analyses, D, C/c and E/e behave like the products of three closely linked genes and the Rh haplotypes are often written as *DCe*, *DcE*, *dce*, (shorthand, *R1*, *R2*, *r*, respectively) ,Molecular genetics has shown that there are only two Rh genes, one encoding D, the other encoding the Cc and Ee antigens, but as the Cc and E e polymorphisms are determined by separate regions of a single gene, the DCE terminology is still [3].

2.2.3-Rh antibodies:

Practically all Rh antibodies result from immunization, naturally occurring Rh antibodies, with exception of anti-E, are rare. Immunization may result from the transfusion of Rh-positive blood into an Rh negative person, or from the passage of Rh positive cells from a fetus into the circulation of an Rh negative mother during pregnancy.

When an Rh-negative person has been immunized either by a transfusion or pregnancy, the transfusion of Rh-positive blood can result in a hemolytic transfusion reaction, which may be fatal.

D is a strong antigen and thus a large proportion of Rh negative persons exposed to Rh-positive cells become immunized.

Transfusion constitutes a more effective stimulus than pregnancy. The antibody to the D antigen (anti D) may occur in two forms: as a saline agglutinating antibody (IgM) and as an incomplete antibody (IgG) the latter is the more common.

The other antigenic of the Rh system are much less antigenic than D, and thus are of less clinical importance. However, occasionally anti E, anti C, anti-c and anti-e develop as a result of transfusion or pregnancy; they may develop in D-positive patient. Their presence can be detected by careful cross matching; their identification requires special laboratory investigation [1]

2.2.4-Probable Rh genotype:

When a person's Rh phenotype is known, the probable genotype can be discerned and its likelihood calculated from known genotype frequencies within the same population.

When probable genotype determinations are carried out, it is very important that the ethnic origin of the person is known; figures for one population will not apply to people of other populations. For example, in white populations, *dce* is 15 times more common than *Dce*, whereas in African populations *Dce* has a slightly higher frequency than *dce*. Consequently, the phenotype D+ C+ c+ E− e+ represents a probable genotype of *DCe*/*dce* in a white person, but of *DCe*/*Dce* in a black person.[3]

2.2.5-Weak D:

In serologic testing, D positive blood is easily identified. Units which are D negative are often retested to rule out a weaker reaction. This was previously referred to as D^u, which has been replaced.[6]. By definition, weak D phenotype is characterized by negative reaction with anti-D reagent at immediate spin (IS), negative reaction after 37 °C incubation, and positive reaction at anti-human globulin (AHG) phase. Weak D phenotype can occur in several ways. In some cases, this phenotype occurs because of an altered surface protein that is more common in people of European descent. An inheritable form also occurs, as a result of a weakened form of the R0 gene. Weak D may also occur as "C in

trans", whereby a C gene is present on the opposite chromosome to a D gene (as in the combination R0r', or "Dce/dCe"). The testing is difficult, since using different anti-D reagents, especially the older polyclonal reagents, may give different results.The practical implication of this is that people with this sub-phenotype will have a product labeled as "D positive" when donating blood. When receiving blood, they are sometimes typed as a "D negative", though this is the subject of some debate. Most "Weak D" patients can receive "D positive" blood without complications.[6] .However, it is important to correctly identify the ones that have to be considered D+ or D−. This is important, since most blood banks have a limited supply of "D negative" blood and the correct transfusion is clinically relevant. In this respect, genotyping of blood group has much simplified this detection of the various variants in the Rh blood group system.[7]

2.2.6-Partial D:

It is important to differentiate weak D (due to a *quantitative* difference in the D antigen) from partial D (due to a *qualitative* difference in the D antigen). Simply put, the weak D phenotype is due to a reduced number of D antigens on a red blood cell. In contrast, the partial D phenotype is due to an alteration in D-epitopes. Thus, in partial D, the number of D antigens is not reduced but the protein structure is altered. These individuals, if alloimmunized to D, can produce an anti-D antibody. Therefore, partial D patients who are donating blood should be labeled as D-positive but, if receiving blood, they should be labeled as D-negative and receive D-negative units. [8]In the past, partial D was called 'D mosaic' or 'D variant.' Different partial D phenotypes are defined by different D epitopes on the outer surface of the red blood cell membrane. more than 30 different partial D phenotypes have been described. [8]

2.2.7-Rh null syndrome:

The individuals lack not only D but all the Rh antigens. They have a type of haemolytic anaemia caused by an abnormal cell membrane. These individuals are more prone to develop anti-D antibody [4].

2.2.8-Rh HDFN:

In Rh(D) HDFN, the Rh-positive firstborn infant of an Rh –negative mother usually is unaffected because the mother has not yet been immunized. During gestation and particularly at delivery, when the placenta separates from the uterus, variable numbers of fetal RBCs enter the maternal circulation. When D antigen is inherited from the father, these fetal cells immunize the mother and stimulate the production of anti-D. Once the mother is immunized to D antigen, all subsequent offspring who inherit the D antigen will be affected.

The maternal anti-D crosses the placenta and binds to the fetal Rh-positive cells The sensitized RBCs are destroyed (hemolyzed) by the fetal monocyte-macrophage system, resulting in anemia.

There are several factors that affect immunization and severity of HDFN, including antigenic exposure, host factors, immunoglobulin class, antibody specificity, and influence of ABO group.

The incidence of the disease caused by anti-D has decreased since 1968 with the introduction of Rh-immune globulin (RhIG).[9]

2.3. Blood transfusion:

Blood transfusion is generally the process of receiving blood or blood products into one's circulation intravenously. Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood but modern medical practice commonly uses only components of the blood, such as red blood cell, white blood cell,plasm, clotting factors, and platelets [10] Historically red blood cell transfusion was considered when the hemoglobin level fell below 100 g/L or hematocrit falls below 30% [11]. Because each unit of blood given carries risks, a trigger level lower than that at 70 to 80 g/L is now usually used as it has been shown to have better patient outcome.) The administration of a single unit of blood is the standard for hospitalized people who are not bleeding, with this treatment then followed with re-assessment and consideration of symptoms and hemoglobin concentration. Patients with poor oxygen saturation may need more blood. [12]

The advisory caution to use blood transfusion only with more severe anemia s in part due to evidence that outcomes are worsened if larger amounts are given[13].) one may consider transfusion for people with symptoms of cardiovascular uch as chest pain or shortness of breath.[11] In cases where patients have low levels of hemoglobin but are cardiovascularly stable, parental iron is a preferred option based on both efficacy and safety. [14]

Other blood products are given where appropriate, such as clotting deficiencies, before a recipient receives a transfusion, compatibility testing between donor and recipient blood must be done. The first step before a transfusion is given is to type and screen the recipient's blood. Typing of recipient's blood determines the ABO and Rh status. The sample is then screened for any alloantibodies that may react with donor blood. [15] It takes about 45 minutes to complete (depending on the method used). The blood bank scientist also checks for special requirements of the patient (e.g. need for washed, irradiated or CMV negative blood) and the history of the patient to see if they have previously identified antibodies and any other serological anomalies.

A positive screen warrants an antibody panel/investigation to determine if it is clinically significant. An antibody panel consists of commercially prepared group O red cell suspensions from donors that have been phenotyped for antigens that correspond to commonly encountered and clinically significant alloantibodies. Donor cells may have homozygous (e.g. K+k−), heterozygous (K+k+) expression or no expression of various antigens (K−k−). The phenotypes of all the donor cells being tested are shown in a chart. The patient's serum is tested against the various donor cells. Based on the reactions of the patient's serum against the donor cells, a pattern will emerge to confirm the presence of one or more antibodies. Not all antibodies are clinically significant (i.e. cause transfusion reactions, HDN, etc.). Once the patient has developed a clinically significant antibody it is vital that the patient receive antigen-negative red blood cells to prevent future transfusion reactions. A direct antiglobulin test (coombs test) is also performed as part of the antibody investigation. [16]

If there is no antibody present, an immediate spin cross match or computer assisted crossmatch is performed where the recipient serum and donor rbc are incubated. In the immediate spin method, two drops of patient serum are tested against a drop of 3–5% suspension of donor cells in a test tube and spun in a serofuge. Agglutination or hemolysis (i.e., positive Coombs test) in the test tube is a positive reaction and the unit should not be transfused.

If an antibody is suspected, potential donor units must first be screened for the corresponding antigen by phenotyping them. Antigen negative units are then tested against the patient plasma using an antiglobulin/indirect crossmatch technique at 37 degrees to enhance reactivity and make the test easier to read.In urgent cases where crossmatching cannot be completed, and the risk of dropping hemoglobin outweighs the risk of transfusing uncrossmatched blood, O-negative blood is used, followed by crossmatch as soon as possible. O-negative is also used for children and women of childbearing age. It is preferable for the laboratory to obtain a pre-transfusion sample in these cases so a type and screen can be performed to determine the actual blood group of the patient and to check for alloantibodies.[16]

2.4.Previous studies:

2.4.1.ABO and Rh frequencies in Sudan:

Several studies have been carried out to determine the frequency of ABO and Rh blood group antigens and phenotypes among the Sudanese ethnic groups. In such study in Elmanaseer tribe, The O-antigen was the most common antigen among the study followed by B,A antigen and the least antigen was AB-antigen, Alleles –e-c-and D- antigens were the most frequent in the study tribe , while C and E antigen were reported at lower frequencies, the Du antigen was very rare [17]

The frequency of ABO and Rh blood groups among Elmanaseer ethnic group table (2-1).

Bloodgroup	Frequency	Percent
A+ve	175	17%
A-ve	1	1%
B+ve	26	26%
B-ve	2	2%
AB+ve	3	3%
AB-ve	1	1%
O+ve	45	45%

Similar study in Elshokria tribe to determine the frequency of ABO and Rh antigens and phenotype, was observed that the most common antigen was O antigen with percent of 58% followed by A-antigen 24% and B-antigen 14% ,AB 4%.The percentage of positive –negative Rh antigens demonstrated as 96 % of the study group were positive –D, while 4% were negative D-antigen [(18)].

Other study in Alshigia tribe revealed that Group O-is the most common antigen in the study group 49%, followed by the incidence of group A-antigen 26%, B-antigen 22%, the incidence of group AB –antigen showed the lowest frequency 3%.The frequent of Rh D-antigen showed that 96% were positive and 4% were negative [(19)]

In other study in AIbderia sudanese tribe to determine the frequency of ABO and –Rh systems ,the O blood group was the most frequent phenotype (51%) ,AB-blood group was the least phenotype in this study with percent of (4%), while A-and B-antigens were (16%) and (32%) respectively .The Rhesus –D was (96%) , -E (18%), -C (66%), -e (91%) and Rhesus –c was (79%) [(20)]

Similar study in Alarakeen ethnic group O-antigen was the common phenotype detected in Alarakeen tribe (40%), followed by –e-c and D were highly frequent among the study group (100%), (96%), (91%), followed by –C and –E (48%), (19.4%) [(21)]

2.4 .2. Frequency of Rh D:

ē was the most frequent antigen among all Sudanese populations (frequencies 95-100%). The majority of the tribes (8/10; 80%) had markedly high c - antigen frequency of 91-100%. With respect to the distribution of D antigen it was observed that six tribes (6/10) had high frequency (92-99%), while the rest high had frequencies ranging from 83-88% . The prevalence of C antigen was less prevalent while the frequency of E antigen was the least common. C antigen was markedly low in the West and South West tribes [Nuba, Denka, Zagawa and Miseria [21]

Tribe	*e*	*c*	*D*	*C*	*E*
Danagla	95	92	94	44	24
Denka	99	100	99	13	17
Halaween	100	84	98	62	22
Hadandwa	96	95	83	48	17
Gaaleen	99	82	84	56	17
Miseria	97	99	83	34	19
Mahas	100	96	88	59	36
Nuba	99	100	92	18	10
Shigia	99	91	94	63	33
Zagawa	100	99	92	19	15

RH Blood group system phenotypes, haplotypes and probable genotypes among major Sudanese tribes table (2-2)

-ABO and Rh blood type distribution by country Table (2-3).

Country	Population	O+	A+	B+	AB+	O−	A−	B−	AB−
Australia[23]	24,642,693	40.0%	31.0%	8.0%	2.0%	9.0%	7.0%	2.0%	1.0%
Austria24]	8,592,470	30.0%	37.0%	12.0%	5.0%	6.0%	7.0%	2.0%	1.0%
Belgium[25]	11,444,053	38.0%	34.0%	8.6%	4.1%	7.0%	6.0%	1.5%	0.8%
Brazil26]	211,248,418	36.0%	34.0%	8.0%	2.5%	9.0%	8.0%	2.0%	0.5%
Canada(27)	36,627,140	39.0%	36.0%	7.6%	2.5%	7.0%	6.0%	1.4%	0.5%
Cyprus(28)	1,189,395	35.22%	40.35%	11.11%	4.72%	3.85%	3.48%	0.87%	0.40%
Czech Republic(29)	10,555,152	27.0%	36.0%	15.0%	7.0%	5.0%	6.0%	3.0%	1.0%
Denmark[30]	5,711,902	35.0%	37.0%	8.0%	4.0%	6.0%	7.0%	2.0%	1.0%
El Salvador(31]	6,171,483	62.0%	23.0%	11.0%	1.0%	1.0%	1.0%	0.7%	0.3%
Estonia(32])	1,305,745	29.5%	30.8%	20.7%	6.3%	4.3%	4.5%	3.0%	0.9%
Finland[33)	5,541,328	27.0%	38.0%	15.0%	7.0%	4.0%	6.0%	2.0%	1.0%
France(34)	64,939,560	36.0%	37.0%	9.0%	3.0%	6.0%	7.0%	1.0%	1.0%
Iceland(35)	334,311	47.6%	26.4%	9.3%	1.6%	8.4%	4.6%	1.7%	0.4%
India(36)	1,342,561,902	27.85%	20.8%	38.14%	8.93%	1.43%	0.57%	1.79%	0.49%
Ireland(37)	4,749,263	47.0%	26.0%	9.0%	2.0%	8.0%	5.0%	2.0%	1.0%
Israel(38)	8,323,659	32.0%	34.0%	17.0%	7.0%	3.0%	4.0%	2.0%	1.0%
Jamaica(39)	2,813,316	47.0%	23.0%	20.0%	3.0%	3.5%	2.0%	1.0%	0.5%
Japan(40)	126,044,340	29.9%	39.8%	19.9%	9.9%	0.15%	0.2%	0.1%	0.05%
Luxemburg(41)	587,297	35.0%	37.0%	9.0%	4.0%	6.0%	6.0%	2.0%	1.0%
Netherlands(42)	17,033,012	39.5%	35.0%	6.7%	2.5%	7.5%	7.0%	1.3%	0.5%

Chapter Three

Materials & Methods

3. Materials and Methods

3.1.Study design:

This descriptive cross sectional study aimed to determine the frequency of ABO and A-sub group among Alrashida Tribe. .

3.2. Study area:

This study was conducted in southern Shendi town during a period of 6 months between March and August, to determine the ABO and Rh phenotypes frequency among Alrashida tribe .

3.3.Study population:

This study included fifty unrelated volunteers from Alrashida tribe at different ages and both sex(male and female).

The consent of selected individual to the study was taken after being informed with all detailed objectives of the study and its health emphasis in the future.

3.4. Inclusion criteria:

a-all age .

b-both sex.

c-belonging to Alrashida tribe.

3.5. Exclusion criteria:

a-all those origin from Sudan.

b- those who are mixed with other Sudanese tribes.

3.6. Research tools:

The questionnaire was specifically designed to obtain information about name, sex ,age, tribe, present of inherited disease, present of disease while sample was drown.

3.7. Materials:

-General equipment and reagent:

-Syringe.

-Cotton and gloves.

-Test tubes .

- EDTA container.

-70% alcohol.

-Tube racks.

-Automatic pipette .

-*Tourniquet.*

-Refrigerator.

-Yellow tips.

3.8. Methods:

3.8.1.Samplig:

-Venous blood was drown after sterilization by 70% alcohol ,needle was used with limited occlusion of the arm by the tourniquet, the blood was added to anticoagulant at ratio of 2-4 ml of blood to the 0.2 ml EDTA and mixed gently.

-Aset of –ABO monoclonal –IgM antisera (anti-A, anti B, anti –A1) was used .

3.9. Principle of ABO grouping:

Atest cell (5% suspension or whole blood) is reacted with anti –A , anti A1 and anti –B antisera , and agglutination reaction with certain antisera will indicates the presence of corresponding antigen . negative reaction indicates the absence of corresponding antigen [22]

3.10.Manual method used:

3.10.1.ABO grouping tube method:

1-Clean dry glass tube was labeled A to antisera –A, B to antisera –B, and A1 to antisera –A1.

2-one drop of whole blood was added to the three tubes .

3- the specific antisera was added according to the label.

4- the blood and antisera were then mixed well and the reaction was read macroscopically and microscopically to identify weak reaction.

3.10.2.Interpretation of results;-

-Positive reaction is indicated by clumping of cells due to presence of the – A or B antigen (agglutination).-Negative reaction is indicated by absence of clumping and the cell appear free due to absent of the –A or B antigen (no agglutination).

3.11. Determination of –D atigen by slide method:

3.11.1.Reagent:

1-monoclonal Igm anti –D.

1-clean dry slide was labeled D to antisera –D.

2-One drop of whole blood was added to the slide.

3-the specific antisera was then added .

4-theblood and antisera were then mixed well and the reaction was read macroscopically and then microscopically to identify weak reaction.

3.11.2.nterpretation of results:

.positive reaction is indicated by clumping of cell due to presence of the –D antigen (agglutination).

.Negative reaction is indicated by absence of clumping and the cells appear free (no agglutination).

3.12. Data analysis:

Data were analyzed by using manual method .

3.13. Ethical Consideration:

-Objectives of the study were explained to all individuals participating in this study.

-The consent was obtained from all participating in this study.

Chapter Four

Results

4-Results

This was descriptive study carried out during six months for determination of frequency of-ABO ,A sub group Rh (D) factor and RH antigens among Alrashida tribe .

Fifty separated blood samples were collected from fifty volunteers (male ,female) table (4-1) in age between (10-75) years old.

The –O antigen was the most common antigen among the study group (52 %) followed by –A1 antigen (28 %) .-B antigen (20%) and other groups –AB ,A2 represented (0%) in study group table (4-2).

D- antigen was most common frequency in the study with percentage of (100%) table (4-3).

Table (4-1) Shows the percent of male and female in the study group.

Sex	Frequency	percent
Male	23	46%
Female	27	54%
Total	50	100%

Table (4-2) Shows the frequency of –ABO blood group and A-sub group among Alrashida tribe:

Blood group	Frequency	Percent %
O	26	52%
A1	14	28%
B	10	20%
AB	0	0%
A2	0	0%
Total	50	100%

Table (4-3) frequency of –D antigen among Alrashida tribe:

D -antigen	Frequency	Percent
Positive (+ve)	50	100%
Negative (-ve)	0	0%
Total	50	100%

Chapter Five

Discussion

Conclusion

Recommendations

5.1-Discussion

This is a descriptive study that was conducted in southern Shendi locality during the period of six months, aimed to determine the frequency of –ABO ,A sub group and –RH systems –CcDEe blood group antigens and phenotypes among Alrashida tribe also it was attempted to compare between Alrashida tribe and different Sudanese tribes and other population , the results besides others on different tribes may help in diagnosis and as reference value used in blood transfusion .

Regarding to ABO blood group system and A –sub group our result reveal that the frequency of O phenotype was the highest frequency (52%) followed by B (20%) .According to A-sub group A1 was the highest frequency (28%) ,the frequency of A2 and AB phenotype not found .

The frequency of O phenotype (52%) was highest than that obtained in Almanasser and Al shigia, Australia[23], Canada [27], France[34] and India [36] was lowest than that obtained in Alshokria , El Salvador[31].

The A phenotype (28%) was highest than that found in Almanasser ,Alshigia and Alshokria Ireland[37)] and Israel[38) .

concern with frequency of B phenotype (20%), it was greater than that found in Alshokria, Austria24], Denmark [30] and Ireland [37] and similar to that found in Jamaica [39] and Japan [40] .

In our study were found that O blood group is the most frequent among Alrashida tribe (52%) , also A1 –sub group is most common.

In the present study when concerning the Rhesus blood group antigens we found that the frequency of D antigen was (100%) which was highest frequency followed by C antigen (62%), no frequencies found for other Rhesus antigens.

In contrast with other Sudanese populations ,the frequency of D antigen (100%) Was agree with the frequency among Denka tribe, and highest than that found in Alshigia, Alshokria and Albederia.

Marked similarities and differences between some Sudanese and other population such as Whites population.

5.2.Conclusion

- From this study we conclude that, there is a difference between the frequencies of ABO blood group antigens in Alrashida tribe and Sudanese tribe.
- O blood group was the most common phenotypes in Alrashida tribe, A2, AB blood groups were the rare phenotypes in this study.
- The rhesus –D antigen was (100%).
- Considerable similarities exist between Alrashida and different Sudanese tribes.
- Our finding confirms that the distribution of blood group in different areas of world varies and this may be explained to be due to genetic differences between different populations.

5.3. Recommendations

- Full Rhesus phenotyping for the common Rh should be performed routinely in the blood banks.
- Rhesus typing should be considered in special cases such as for the pregnant women, previous transfused patient, and patient with known irregular antibodies
- It is necessary to determine blood groups of different ethnic groups and geographical areas to be useful in blood transfusion using large size.
- Study the correlation between the blood groups and some disease that are common in Alrashida community.
- Determination of other blood group system rather than that included in this study in Alrashida ethnic group.
- Stable records for all blood groups should designed in Alrashida tribe to minimized and avoid blood transfusion reactions.

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Appendix

Appendix I

Shendi University

Collage of Graduate Studies and Scientific Research

Questionnaire:

Determination of ABO and Rhesus Blood D antigen phenotypes among Alrashida ethnic Group

. Name: ……

. Age :……..

. Sex :……..

.tribe : ……....

.Present of inherited disease: …….

. Present of disease while the sample was drown……..

. **Result:**

ABO grouping antigens:

Ag	A1	A	B	AB	O
Result					

Rh antigens:

Ag	D
Result	

Date:……..

Signature:…….

Appendix II

إقـــرار بالموافقـــة

الاسم :----------

العمر :---------- العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة فصائل الدم لدى قبيلة الرشايدة.

الباحثة: نهى إبراهيم حسن ناصر

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر عليَّ بأي حال من الأحوال كما أنه يحق لي بدون إبداء أسباب الانـــسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشـــــــراف:

د. حمزة احمد حسن

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