Sudan University of Science and Technology

College of Graduate Studies

Frequency of ABO blood Groups and Sub group A among Eljaleen tribe in Khartoum State

قياس نسب فصائل الدم و المستضادات الفرعية للفصيلة A بين أفراد قبيلة الجعلين في ولاية الخرطوم

A dissertation Submitted for Partial Fulfillment for the degree of M.Sc in Hematology and Immunohematology

Submitted by

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بسم الله الرحمن الرحيم

الآية

قال تعالي :

إِنَّمَا أَمْرُهُ إِذَا أَرَادَ شَيْئًا أَن يَقُولَ لَهُ كُن فَيَكُونُ ﴿٨٢﴾ فَسُبْحَانَ الَّذِي بِيَدِهِ مَلَكُوتُ كُلِّ شَيْءٍ وَإِلَيْهِ تُرْجَعُونَ ﴿٨٣﴾

صدق الله العظيم
سورة يس
الايات (82-83)

Dedication

At the end of this stage

I would like to dedicate this study to

Soul of my father and

my mother

Because they are always with me by their support

during my life

Acknowledgment

I would like to thank our God for helping and supporting

I would like to thank Sudan University of Science and Technology Department of Hematology

Greatest thanks for Dr. Monsour Mohmmed Monsour

Also I would like to thank anyone

who help me in this study

Abstract

Introduction: there are differences in the distribution of ABO blood group and subgroup A in different population in the world .Relatively little information is available about blood group distributions in Sudanese population .This was across sectional study conducted in Khartoum state to assess the distribution of ABO and subgroup A in four months (January to April 2014) among the study subject

Material and metod :Two hundreds unrelated individuals unrespectable to sex from Eljaleen tribe were enrolled for this study. The investigations were done using finger prick ,slide methods and analyzed by SPSS computer program.

Results: this study revealed that blood group O was the most predominant (40%) followed by blood group A (34%), blood group B was (22%)and blood group AB which represented (4%). Among the study group prevalence of subgroup A was (89.71%)and prevalence of other subgroups was (10.2 9%).

Conclusion: This study concluded that distribution of ABO blood groups and subgroup A among ELjaleen tribe was not or was similar to its distribution to major other Sudanese tribes.

ملخص البحث

الدراسة: هناك اختلافات في توزيع فئات الدم ABO و المستضادات الفرعية A في السكان في العالم . يوجد القليل من المعلومات المتاحة عن توزيعات نسبة فئات الدم و مستضاداتها الفرعيه في سكان السودان ، اجريت هذه الدراسة المقطعية في ولاية الخرطوم لتقييم توزيع فئات الدم ABO و المستضادات الفرعية للفئة Aفي غضون أربعة أشهر (من يناير حتي ابريل 2014) في أفراد من قبيلة الجعلين لا تربطهم علاقة ببعضهم البعض.

الطريقة: تم اخذ مائتي عينه من الدم الطرفي من الذكور والاناث ،تم تحليلها باستخدام طريقة الشريحة البسيطة الشريحة وتحليلها بواسطة برنامج التحليل الاحصائي للمجتمع .

النتائج: كشفت نتائج هذه الدراسة أن فئة الدم O كانت أكثر غالبية (40 ٪) تليها فئة الدمA(34%) ، وكانت فئة الدم B (22٪) و فصيلة الدم AB والتي تمثل (4 ٪) . بين انتشار مجموعة الفئات الفرعية A1 (89.71 ٪) و كان معدل انتشار الفئات الفرعية الأخرى (10.29 ٪) .

الخلاصة :اثبتت هذه الدراسة أن توزيع فصيلة الدم ABO والمستضادات الفرعية للفصيلة A في قبيلة الجعلين مماثلا لتوزيعها على القبائل السودانية الأخرى الرئيسية.

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Abbreviations

DNA:Deoxy Ribo Nucleic Acid

FMH: Feto Maternal Hemorrhage

HDFN:Haemolytic Disease of fetus and New born
IAT:Indirect Antihuman globulin Test

IgG:Immuno globulin Gamma

IgM:Immuno globulin μ

LISS:Low Ionic Saline Solution

Rh:Rhesus antigens

SPSS:Statistical Package for Social Sience

Chapter one

Introduction and literature review

1-1 Introduction

Blood group serology include the study of antigenic molecules present in the various cellular and soluble component of whole blood together with that of the antibodies and lectins that recognize them and their interaction . however in practice blood group serology is restricted to RBCs antigens and their interaction with specific antibodies .There are several blood group systems to which antigens present on the surface of the RBCs .Approximately 400 RBCs antigens have been described (Hoffbrand and PPettit,2001). Blood group are quietly different between the different ethnic groups throughout the world .Since Sudanese ethnic are emigrating from different areas they expected to have different complex distributed blood group system. This study could also used to determine the ethnic interaction between the major ethnic group (Hoffbrand and PPettit,2001).

1-2 Literature Review

1-2-1 Historic Perspective ABO system

Karl Landsteiner truly opened the doors of blood banking with his discovery of the first human blood group system, ABO this market the beginning of the concept of, individual uniqueness defined by the red cell antigens present on the red cell membrane. The ABO system still remains the most important of all Blood groups in transfusion practice. Transfusion of an incorrect ABO type can result in death of the patient. In 1901 Landsteiner drew blood from himself and five associates. Separated the cells and the, serum and then mixed each cell sample with each serum. He was inadvertently the first individual to perform forward and reverse groupings**.** Forward grouping is defined as using known sources of reagent antisera (antibodies) to detect antigens on an individual's red cell. Reverse grouping is defined as using cell reagent with known ABO Antigens and testing the serum of the patient for ABO group Antibodies (Lewis and Barbra 2001). Groups A, B and O were the first blood group described by Landsteiner. He found that serum from group B individuals agglutinated group A red Blood cells and therefore, that an antibody to Antigens was present in group B serum. Conversely, serum from group A individuals agglutinated group B red cells and therefore, an antibody to B antigens was present in group A serum. Serum from group O individuals

agglutinated both A and B cells, indicating the presence of antibodies to both A and B antigens (Lewis and Barbra 2001).

Table 1-1 ABO for ward grouping (Lewis and Barbra 2001)

Patient'sred cells	Reaction with reagent (Al),	Reaction with Anti (B)	Interpretation of blood group
1	Negative	Negative	O
2	positive	Negative	A
3	Negative	Positive	B
4	positive	Positive	AB

Table 1-2 ABO for reverse grouping (Lewis and Barbra 2001)

Patients serum	Reaction with A red cells,	Reaction with B red cells	Interpretation of blood group
1	+	+	O
2	Negative	+	A
3	+	Negative	B
4	Negative	Negative	AB

In 1902 Landsteiner's associates, Sturle and von Decastello discovello, discovered the fourth ABO blood group, AB, As can be seen from table (1-2) serum from group AB individuals does not agglutinate group A or group B cells indicating the absence of antibodies to both A and B. The frequency of these blood groups in the white population is as follows: group O 45%, group A 41%, group B 10 % and group AB 4 %, therefore O and A are the most common blood group types. And blood group AB the rarest, however, frequencies of ABO groups differ in a few selected populations for example group B is found twice as frequently in blacks and Asians as in whites, subgroup A_2 is rarely found in Asians**.** Landsteiner concluded from the reactions he observed that in the ABO blood group system individuals have naturally occurring antibodies in their serum directed against the missing ABO antigen on the surface of their red blood cell. This term "naturally occurring is really a misnomer, inasmuch as substantial evidence suggests that anti AB and anti B are stimulated by substance that are ubiquitous in nature. Bacteria have been shown to be chemically similar to human ABO antigens and may serve as source of stimulation of antibody formation. ABO Antibodies are a result of a cross-reactivity and are initiated at birth upon exposure to foreign substances ubiquitous in nature(Lewis and Barbra 2001).

Titers are usually low for detection unit the individual is 3 to 6 months old. As a result it is logical to perform only forward grouping on cord

blood from newborn infants. The antibody production peak when the individual is *5* to 10 years old and then declines progressively with advanced age. Patients older than Ag 65 usually have low titers, so that antibodies may be undetectable in the reverse grouping. The ABO blood group system is unique in that all normal, healthy individuals consistently have present in their serum antibodies to antigens they lack on red cells. Testing of ABO is relatively easy therefore, the regular occurrence of Anti A or Anti B or both in persons lacking the corresponding antigens serves as a confirmation of results in the forward grouping. Complete absence of Anti A and Anti B is very rare in healthy individuals (except in AB subjects) occurring at less than 0.01 percent in a random population. If group B or A red cells are given to a patient whose serum contains anti B or anti A, the donor's red cells will be destroyed almost immediately, being lyses at a rate of approximately 1ml of red cell per minute this produces very severe if not fatal transfusion reaction in the patient, therefore both forward and reverse grouping must be performed on all patient samples noting the correct reciprocal relationship of Antigens and antibodies in a given blood group type **(**Lewis and Barbra 2001).

Table 1-3 summary of forward and reverse grouping (Lewis and Barbra 2001)

Patient cells tested with			Patient serum tested with		
Anti A	Anti B	Interpretation	A cells	B cells	Interpretation
negative	negative	O	Positive	Positive	O
positive	negative	A	Negative	Positive	A
negative	positive	B	Positive	Negative	B
positive	positive	AB	Negative	Negative	AB

1-2-2 ABO blood group system and ABO variant

In 1875 Lando is first noticed clumping agglutination when red cell of animals of one species is treated with serum of another species. In Human being the same phenomena was observed by Karl Landsteiner in 1900 that lead to discovery of ABO blood groups, later Decastillo and Sturle (students of Landsteiner) discovered another group in Human blood and that is AB. And thus the whole of human population was divided in four groups on the basis of major blood groups to which they belong. In 1910 Dungerin and Hirsz field established the Mendelian Inheritance of blood group and was later confirmed in 1924 by math matician Bernstin (Lewis and Barbra,2001).

Other systems of blood group were rediscovered after about *25* years of discovery of ABO system. As has been stated above an individual Us blood group is determined by genetic control governed by principles of Mendelian inheritance. Supplemented by Landstiner's law and regular presence of ABH antigens in tissue cell and secretions in the secretors. Landsteinar law states that there is a reciprocal relationship between antibody present in serum / plasma and antigen on his own red cells. In his experiment he observed that a person serum does not contain the antibody against the antigen present on his own red cells via individual with A antigen on red cells will not contain anti A in the serum instead it contain Anti B. He further observed that two Antigens namely A and B were needed to explain four groups (A, B, O and AB) in whole human population. Blood groups are the phenotype expression of individual but the genetic constitution in genotype is expressed in a different way (Lewis and Barbra 2001).

Table 1- 4 phenotype and genotype(Lewis and Barbra 2001)

Name of phenotype	Genetic constitution or genotype
O	OO
A	AA or AO
B	BB or BO
AB	AB

The group A is further subdivided in A1 and A2 known as sub groups of A and therefore increase the number of phenotypes from four to six A1, A2, A1B and O this lead to further experiment and it was observed that anti A obtained from serum of group B persons has two antibodies namely anti A and blood group A1 has two antigens on red cells A and A1 where as A2 has only one antigen namely A. So the distribution of antigens on red cells and antibodies in serum / plasma respectively present in six blood group A1 , A2, A1B, A2B,B, and O can be plotted as follow table 1-5 (Lewis and Barbra,2001).

Table 1-5 type of antibodies in Blood Group (LewisS.Mand Barbra 2001)

Antibody	Type of antibody	Antigen agglutinogen	Blood group
Anti B	Usually 1g M	A and A_1	A_1
Anti B	Usually 1 g M	A only	A_2
None	X	A and A_1 and B	A_1B
None	X	A and B	A_2B
Anti A and Anti A_1	Usually 1 g M	B	B
Anti A, Anti A_1 and Anti B	Usually 1 g G	None	O

Weaker variants of A and subsequently for group B were discovered and were classified on the base of A and B antigens in the saliva of secretors mainly classified in Ax / Bx and Am /Bm. The Am/ Bm type possesses A/B and H while Ax/ Bx possesses only H in saliva, weaker variants of A discovered till now are (Al,A2, A3, Ax and Am), weaker variants of B so ‘far reported are (B3, Bx, Bm) But all these subgroups or weaker variants reported so far except Al and A2 are more of theoretical value (Lewis and Barbra,2001).

1-2-3 Importance of ABO Group

The importance of a blood group system in Blood transfusion lies in the frequency of its antibodies and in the possibility that such antibodies will destroy incompatible cells in vivo. The ABO system was the first to be recognized and remains the most important. The reason for this is that almost everybody over the age of about 6 months has clinically significant Anti A and / or anti B in their serum if they lack the corresponding Antigens on their red cells. Although the importance of ABO antigens in relation to transfusion are also expressed on most endothelial and epithelial membranes and are important histocompatibility antigens ,transplantation of ABO incompatible solid organs increases the potential for hyper acute graft rejection. Major ABO incompatible stem cell transplant (e.g. group A stem cells into group O

recipient) will provoke haemolysis, unless the donation is depleted of red cells (Tolil and Dutta 1995)

1-2-3-1 Biochemical structure of ABO system

This consists of three allelic genes A, B, and O. The A and B genes control the synthesis of specific enzyme responsible for the addition of single carbohydrate residues, N-acetyl glactosamine for group A and D-glactose for group B to a basic Antigenic glycoprotein or glycolipid with a terminal sugar (L fucose on red cell, known as H substance) the O gene is an a morph and does not transform the H substance. The two major subgroups of A (Al and A2) complicate the issue but are of minor clinical significance A2 cells react more weakly than A1 cells with anti A and patients who are A2B can be wrongly grouped as blood group B. The A , B and H antigens are present on most body cell including white cells and platelets in the 80% of the population who posses secretor genes , this antigen, are also found in soluble form in secretions and body fluids e.g. plasma, saliva, semen and sweat. Naturally occurring antibodies to A and\ or B antigens are found in the plasma of subjects whose red cells lack the corresponding antigen. They are mainly A, B and H antigen which are protein in nature and various proteins are

embedded inamozaic pattern without any fixed position on fluid lipid layer of cell membrane (Singer and Nicholson 1972) but Marchase in 1979 proposed that spetrin forms the core of the matrix of closely packed units of various form via rod like, they interacting with transmembrane proteins forms a complex of fixed substrate and thereby allowing a restricted movement of the protein only on the cell membrane. These proteins on red cell membrane are classified as:

A. peripheral which can be dissociated readily.

B. Integral that cannot be dissociated easily and has tendency to remain associated with lipids even after drastic treatment. The A, B and H antigen sites are greatest on band 3 of sialogly co protein and they are also found on polyglycosil ceramides and simple glycolipid. The number of A, B and H antigen sites varies in new born and adults. These antigenic sites are important because the antibody molecules get attached to red cells at these sites. In adults A1 varies between 810,000 to 1170,000 and in new born 250,000 to 370,000 where as in A2, adults it varies between 240,000 to 290,000 and in new born 140,000. In B adults it is approximately around 750,000 and in new born 220,000.in A1B adult 460,000 to 850,000 in new born 220000 to 320.000 A2B adult 120.000 to 140.000 H sites on O cells in new born is 325,00 and in adult is 1700,000 but in new born A, Band AB sites approximately around 70,000. So that been observed that the order of reactivity of H antigenic

determinant is nil in Bombay phenotype and progressively reduces as follow O> A2>A2B>A1> A1B therefore O red cells contains the numerically greatest number of H antigenic site. The ABH antigens are widely distributed they are even found in animals plants and bacteria. The A and B antigens occur on platelets, white cells and serum. A tremendous volume of biochemical works were done to determine the chemical nature of AB and H antigens and their genetic back ground. A simple and brief description is follows. A,B and H antigens can exist as glycoprotein in secretion but in cell membrane it exists as glycolipid .The basic difference in chemical reactivity of glycolipid and glycoprotein respectively shows that glycolipid are alcohol soluble and could be extracted by treatment with alcohol only subsequently it was observed that antigens are highly branched complex of glycosphingolipids. Lipid part of which remains attached to red cell membrane where as protein part exposed above is responsible for antigenic specificity. Glycoprotein are soluble antigen part exposed in at least 75 to 78% Individuals in there tissue and body secretion. Glycoprotein are water soluble and specificity corresponds to individuals own blood group. Their presence was detected in almost all body secretion except cerebrospinal fluid. They have been traced to saliva, urine, bile sweat, tears, serum and gastric juice. The persons in whom blood group substance is found are called secretors and whom it is not found are called non-secretors. The polysaccharide protein

of glycoprotein and glycosphingolipid are similar and the chemical constitution of two basic chains reveals presence of L. fucose, N- acetyL D-galactosamine, N-acetyL, D-glucosamine, D glucose. The difference between type 1 chain and type2 chain that in the former the terminal galactose is bind by B (1-3) linkage where as in the later the galactose is bound B 1-4 linkage . The antigenic specificity is determined by the terminal sugars via fucose when attached to the basic chain the antigenic specificity is H, if it is N-acetyL-galactosamine the specificity is A and if it is D- galactose the antigenic specificity is B. The attachment to basic chain is effected through enzyme tranferase precursor chain is substrate for H genes specified fucosyL tranferse with addition of sugars to basic chain anew structure is formed. This acts as substrate for another enzyme transferase for A specificity. N-acetyl, D-galactosamine is added to H chain by A gene specified transferase and so on (Harmening Denise 2005).

The ABO blood group systems are inherited, the system consists of a single genetic locus from where three allelic genes namely A, B and O operates. A gene can be Al and A2. following table 1-6 is expanded version. A and B genes are codominant and O is an amorph(gene does not express phenotypically) since each body cell has a pair of chromosome and each of which either carries or fails to carry one of the above genes the genotypic constitution will be represented as above in the table 1-6 .

The thing that is detected in blood are not gene but the product produced under appropriate genetic influence and that is the observed trait or phenotype of individual . In heritance of blood group as a result of mating between parents is correct it has been shown mathematically in the table given below table 1-6 (Harmening Denise 2005).

Table 1-6 genotype and phenotype of ABO **(**Hoffbrand and PPettit,2001)

Phenotype	Genotype
A	AA and AO
B	BB and BO
AB	AB
O	OO
A_1B	A_1B
A_2B	A_2B
A_1	A_1A_1 , A_1A_2, A_1A_3 , A_1A_4 , A_1 O
A_2	A_2A_2 , A_2A_3 , A_2A_4, A_2O
A_3	A_3A_3, A_3A_4 , A_2O
A_4	A_4A_4,A_4O
A_3B	A_3B

A_4B	A_4B

One the paregenes transmissible by one parent and on left side by other parent. All the alleles present in one locus may not have the same frequency because variations with in species may result from genetic change which is unnatural this phenomena known as mutation. This when occur in any individual of apopulation ultimately gives rise to allelism and subsequently through mating over long period new allele gets distributed into the particular group to which the individual originally belonged **(**Hoffbrand and PPettit,2001).

The percentage of occurrence of all genes of particular trait called gene frequency and sum total of which must be equal to 100% from the fore going statement and the tables it is thus clear that one of the three possible genes is transmitted by each parent to their off spring through mating. Combination of two separate genes respectively from each parent results in formation of genotypic constitutions.This can be detected in the reation by treatment with antisera A and B ,the reaction exhibits dosage effect if genetic constitution of individual is homozygous . in homozygous via DD the reaction will be stronger with antisera D than on homozygous via Dd**.** In addition to red cells, ABH antigens can be found in epithelium tissues, organs such as bone marrow and kidneys, lymphocytes and platelets. It has recently been reported that (ABH)

antigens are not present on granulocytes. ABH soluble antigens can be synthesized and secreted can be found in all the body secretions , depending on the ABO genes inherited as well as on the inheritance of another set of genes (secretor genes) that regulates their formation **(**Hoffbrand and PPettit,2001).

1-2-3-2 inheritance of ABO

In normal people, the nucleus of all somatic cells contains 46 chromosomes arranged in 23 pairs 22 pairs of autosomes and one pair of sex chromosomes. Inherited characteristics such as blood group antigens are controlled by genes, which are carried on these chromosomes. Each chromosome of pair (homologous chromosomes) contains genes that affect the same characters and these are arranged in a similar order on both chromosomes. In the germ cells a process of reduction division (meiosis) reduces the chromosomes to 23, so that in the ova and in the germ cells a process of reduction division (meiosis) reduces the chromosomes to 23 so that in the ova and in the spermatozoa there is only one representative chromosome from each pair. This is the haploid state fusion of the ovum and the spermatozoa leads to pairing of their chromosomes so that each inherited characteristic in the new individual is controlled by at least two genes, one from the mother and from the mother and one from the father. The pair of gene that controls a single character can be same or different. Alternative genes occupying the same

locus or site, on the chromosome are known as alleles or allelomorphic genes. If the two gene for a character are identical, the person is said to be homozygous e.g. DD for that character. If the two genes are different the person is said to be heterozygous e.g Dd (Garratty *et,al*.2000).

1-2-3-3 ABO Chromosomes mapping and Linkage

Characteristic found to be associated through several generations are considered to be governed by gene within a measurable distance of each other on the same chromosome linkage. The nearer the loci, the closer the linkage. Linked loci at some distance from each other may be separated by crossing over which occurs at the first meiotic division. Alleles at loci on different chromosomes or alleles far apart on the same chromosome segregate independently. The terms cis and trans help to describe the positional relationship of genes to each other gene occupying loci on the same chromosome are positioned cis to one another while genes on opposite chromosomes of a pair are positioned trans to each to each other. AB phenotype and genotype Blood group antigens are stable characteristics controlled by genes inherited in a simple Mendelian manner. Serological techniques do not test for the presence or absence of gene itself, but for the gene product. Agene is expressed when its product can be observed. Genes expressed only in the homozygous state are recessive. Gene expressed in the heterozygous state in the presence or absence of expression of their allele are dominate co dominant is the state

in which two different alleles are equally expressed. **(**Hoffbrand and PPettit,2001).

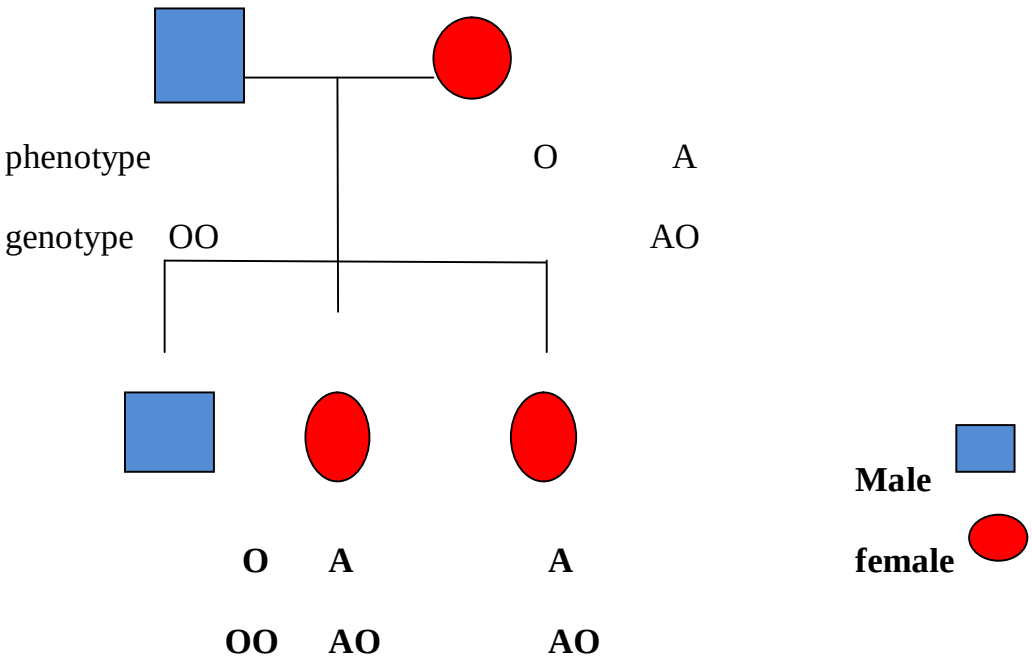


Fig (1-1) a family study from which the ABO Genotypes of all the family members can be determined. **(**Hoffbrand and PPettit,2001)

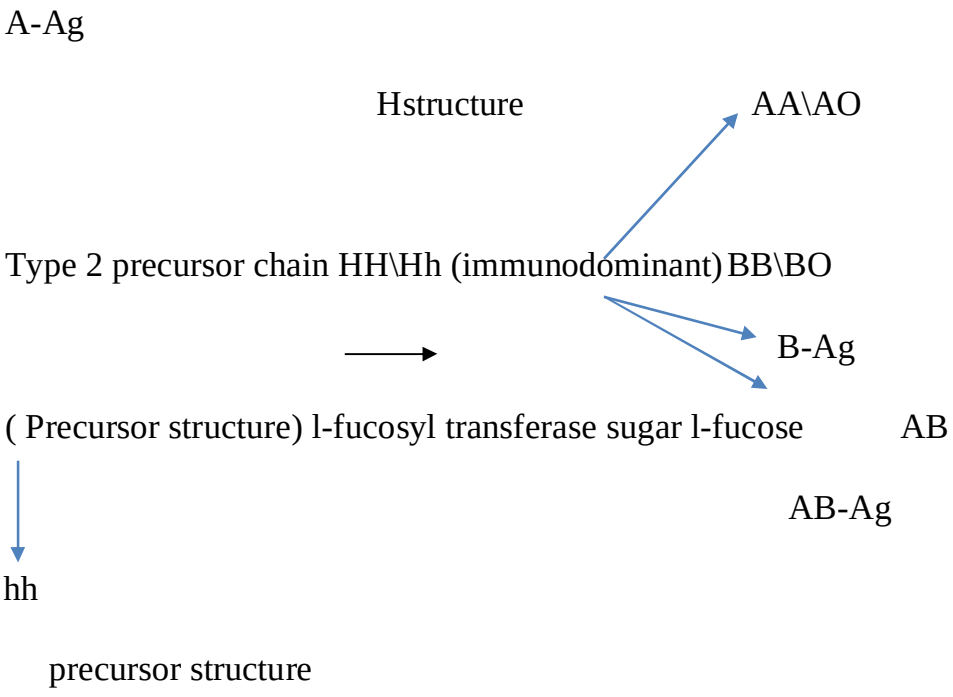
1-2-4 Genetic of ABO system

Some blood group antigens, if proteins are the direct product of the pertinent gene e.g. Rh other especially carbohydrates e.g. ABO result from the action of enzymes transferases on the appropriate substrate or precursor substance. These substrates are usually the product of genes located at unlinked loci for example , H the product of the H genes chromosome 19 can be converted to A or B by the action of the A or B transferees chromosome 9 if gene coding for the precursor substances are absent, the final blood group antigen will not be expressed, even if the appropriate genes coding for the final product are present, for example, in the absence of the H gene , A or B cannot be expressed since no substrate is available for the relevant transferes (Millison PL,1999).

1-2-4-1 Interaction of secretor and (ABH) genes

It is believed that two other genetic systems control the expression of ABH genes. More specifically, they control the H gene, which in turn, influences the A and B genes. The secretor system was inherited independently of the ABO and H genes. Approximately 78 percent of the ABO random USA population has inherited the Se gene possessing the genotype SeSe or Sese, given the name secretor. The Sese system regulates the formation of H antigen and subsequently of A and B antigens in secretary cells. The secreted A, B and / or H antigens are glycoprotein, as opposed to glycolipids on red blood cell surfaces people who inherit sese are termed non secretors. The exact mechanism of how

these gene functions to regulate tissue secretary cells is not known. However, the H specific transferees (L-fucosyL transferase) is found only in the secretions of secretors, indicating that the secretor gene controls the expression of the H gene in the secretary cells. The Se gene does not however, affect the formation of A, B or H antigens on the red cell and does not control the presence of A, B or H transferase in hemopoietic tissue. In fact A or B transferanse enzymes, unlike A or B glycoprotein substance or antigens are founding the secretions of A or B individuals regardless of their secretor status. However it is the presence of the H gene specified (L-fucosyL transferase) which depends on the inheritance of the Se gene that determines whether ABH -soluble substances will be secreted, in as much as H-substances must be synthesized before the formation of A or B substances **(**Hoffbrand and PPettit,2001).



unchanged (Bombay phenotype)

Fig (1-2) interaction of the Hh and ABO genes **(**Hoffbrand and PPettit,2001

1-2-5 Distinction of A,B and H antigens and AB souble substances

The formation of soluble A, B and H substance is the same as described for the formation of A, B and H antigens on the red cells except for a few minor distinctions:

1. The secreted substances are glycoprotein. The red cell antigens are glycolipids.

2. The first sugar in the common carbohydrate residue of the precursor substance is N-acetylglactosamine for the glycoprotein secretions, and glucose for the red cell antigens. **(**Hoffbrand and PPettit,2001)

3. In the biosynthesis of the glycoprotein secretions, both type 1 and type 2 linkages occur in the precursor structures. In the case of the red cell ABH glycolipid antigens. Only type 2 precursor chain are involved note that type 1 chain refers to a beta 1-3 linkage in which the number one carbon of the glactose is attached number N-acetyl glucosamine sugar of the precursor substance as opposed to a beta 1-4 linkage previously described for a type 2 chain. **(**Hoffbrand and PPettit,2001)

4. In as much as the H structure or substance is the precursor or acceptor substrate for sugar transferred by the A or B gene specified enzymes ., the

Sese system regulates the H- gene activity in secretion but not in the red cell. **(**Hoffbrand and PPettit,2001).

Tab1e 1-7 ABH substance in the saliva of the secretors and non secretors **(**Hoffbrand and PPettit,2001)

ABO group	A	B	H
O	None	None	
A	↑↑	None	↑↑
B	None	↑↑	↑↑
AB	↑↑	↑↑	

Tests for ABH secretion may establish the true ABO group of an individual whose red cell antigens are poorly developed. The demonstration of the A, B and H substances in saliva is evidence for the inheritance of An A gene, a B gene an H gene and an se gene. the term secretor refers only to secretion of A, B or H. soluble antigens in body fluids (hoffbrand and PPettit) .

Table 1-8 ABH- soluble substances in body fluid of secretors **(**Hoffbrand and PPettit,2001)

Saliva	Milk
Tears	Amniotic fluid
Urine	Pathologic fluid = pleural peritoneal,
Digestive juices	Pericardial ovarian cyst.

1-2-6 Antibodies of ABO system

Anti A and B are present in blood when the crossponding antigen is absent on the red cells i.e. they are ciprocal relationship known as Landsteiner's low. Exception to this is very rare and usually found in conditions those are physiologically not normal viz hypogamma globulinemia, dispend or in Am phenotype. In new born and young infants child develops anti body between 3 to 6 months and the limit of normal range is reached by 5 to 11 years. So blood group antibody present in new born believed to have been acquired from mother passively **(**Lewis and Barbra,2001).

The blood group antibody can be mainly subdivided into natural and immune antibody. Natural antibody are IgM in nature and usually acquired from surrounding environment ,genetic factors have no direct control over the titer of antibody. They display homogeneity in physical, chemical property and serological specificity. Immune antibody may develop due to pregnancy, injections with intention to immunize or produce strong blood group system , horse serum , Influenza virus vaccine. And incompatible blood transfusion, plasma transfusion, etc... Immune antibody shows marked heterogeneity which is in contradistinction to natural antibody which displays homogeneity (Lewis and Barbra,2001)

1-2-6-1 Antibody A

This is found in group B and O individuals It is usually 1gM or partly 1gM and partly IgG. It has been observed that after injection of A substances, a group O individual develops additional IgG although the serum initially contained IgM only and it can thus be concluded that development of IgG to anti A and anti B or A individual .The Anti A antibody is usually saline reacting and reacts well with sub groups Al and A2 but the other weaker variants reaction is less defined. Minimal concentration to produce reaction agglutination is only 25 IgM anti A molecules per cell are required but for IgG anti A. it was observed that the number per cell approximates around 20,000 Economidouetal observed that minimal concentration in serum required for IgG is 0.21ug/ml where as for IgM anti A it is 0.01 pg/mL. Anti A can also occur as auto agglutinin and reacts usually weakly at room temperature it may or may not be associated with auto immune hemolytic anemia (John DR,1996)

1-2-6-2 Antibody B

This antibody in found in group A and group O individuals mostly of IgM Type but may be IgG or IgA even. It reacts optimally in saline and exhibits reaction in vitro both by agglutination and haemolysis and IgM reacts more strongly at room temperature and 4°c than 37°c where as immune of IgG B shows better reaction at around 37°c. Economidou *et.al* observed that monomers of 1gM anti B shows. Equilibrium constant 36 -

170 times lower than whole 1gM molecule providing support that native 1gM anti B is attached to red cells by at least two binding sites. Anti B can occur as auto

agglutinin and may show specificity of anti B and reactive at temperature 4°c to 20°c only ,invitro.

1-2-6-3 Cross reacting A and B

It is found mainly in group O sera possess both A and B antibod agglutinins. The cross reacting antibodies reacts with both A cells and B cells. Two different views are had A cross reacting antibodies are directed not only against the specific A or B groupings on the molecule but also against some of common structure on the molecule. if we examine the A or B sugar molecule we will find that they are almost identical save for the difference in 2 position (John ,1996).

1-2-6-4 AntibodyA1and antibody-H

Anti-A1 reacts only with A1 and A1B cells and is occasionally found in the serum of group A2 individuals 1-8% and not uncommonly in the serum of group A2B subjects 25-50%. However, antibody-A1 normally acts as a cold agglutinin and is very rarely reactive at 37°c, when it is only capable of limited red cell destruction. There have been a few reports of red cell haemolysis ascribed to antibody-A1 which some anthers have questioned since, although the antibodies reacted only with

Al red cells, which would have revealed their anti-specificity. Antibody-H reacts most strongly with group O and A2 red cells and also normally acts as cold agglutinin. Notable, but rare, exception is the anti-H that occurs in the Oh Bombay phenotype, which is an IgM anti body and causes lysis at 37°c.(Tolil, 1995**)**

1-2-7 ABO antigens and encoding genes

There are four main blood groups: A, B, AB and O in the British Gaucasian population, the frequency of group A is 42% , B 9% AB3% and O46% but there is racial variation in these frequencies the epitops of ABO antigens are determined by carbohydrates (sugars), which are liked either to polypeptides forming glycoproteins or to lipids glycolipids **(**Hoffbrand and PPettit,2001).

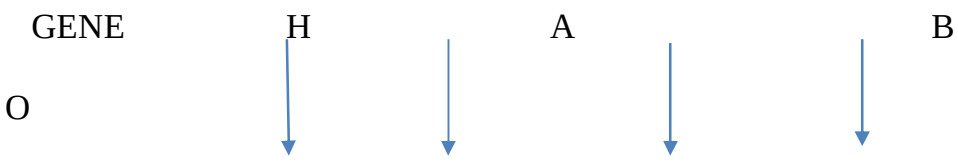
The expression of ABO antigens controlled by three separate genetic loci ABO located on chromosome 9, and FUT2 Se, both of which are located on chromosome19. The genes from each locus are inherited, in pairs as Mendelion dominants. Each gene codes for a different enzyme glycosyl transferase which attaches specific monosaccharide on to precursor disaccharide chains. There are four types of disaccharide chains known to occur on red cells , other tissues and secretions **(**Hoffbrand and PPettit, 2001).

The type 1 disaccharide chain is found in plasma and secretions and is the substrate for the FUT2 Se gene, where as type 2 , 3 and 4 chains are

only found on red cells and are the substrate for FUT1H gene. It is likely that the O and B genes are mutations of the A gene. The O gene does not encode for the production of functional enzymes group O individuals commonly have

deletion at nucleotide 261 which results in a frame -shift, premature termination of the translated poly peptide and the production of an enzyme with no catalytic activity. The B gene differs from A by consistent nucleotide substitutions. The expression of A and B anti genes is determined by H and Se genes which both give rise to glycosyl transferases that add fucose , producing the H antigen The presence of an A or B gene or both results in production of further glycosyl transferases which convert H substance into A and B antigens by the terminal addition of N-acetyl, D-galactos amine and D-galactose respectively since the agene produces an inactive transferase, H Substance persists unchanged as group O. In the extremely rare Oh Bombay phenotype, the individual is homozygous for the h allele of FUTI and hence cannot form the H precursor of the A and B antigen. Their red cells type as group O but their plasma contains antibody-H, in addition to antibody-A, antibody-B and antibody-AB which are all active at 37°c. As a consequence, individuals with an Oh Bombay phenotype can only be safety transfused with other Oh red cells. Serologists have defined two common subgroups of the A antigen, approximately 20% of group A and group AB individuals belong

to group A2 and group A2B respectively, the remainder belonging to group A1 and group A1B . These subgroups arise as a result of inheritance of either the A1 or A2 alleles. The A2 transferase is less efficient in transferring Nacetyl. Galactosamine to available H antigen sites and cannot utilize types 3 and 4 disaccharide chains. As a consequence, A2 red cells have fewer A antigen sites than A1 cells and the plasma of group A2 and group A2B individuals may also contain antibodyA1. The distinction Between these subgroups is most conveniently made using the lectin Dolichos biflorus which only reacts with A1 cells the H antigen content of red cells depends on the ABO group and when assessed by agglutination reactions with antibody-H, the strength of reaction tends to be graded O> A2 >A2B>B>A1>A1B . Other subgroups of A are occasionally found (e.g. A3, Ax). Which are due to mutant farms of the glycosyltransferase transferring N-acetyl -D-galactosamine on to H substance. The A1B and H antigens are detectable early in fetal life, but are not fully developed on the red cells at birth. The number of antigen sites reaches , adult level are around 1 year of age and remains constant until old age when alight reduction may occur (Hoffbrand and PPettit, 2001) .



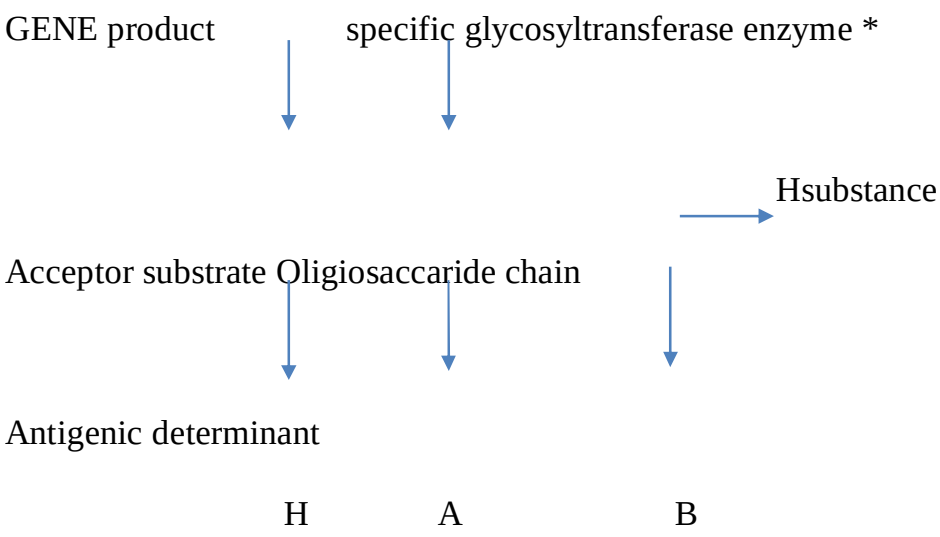


Fig 1-3 pathway from HAB blood group genes to antigens *glycosyl transferase H transfers -L- fucose A transfers N-acetyl -D-glactosamine B transfers D. glactose O is in active (Hoffbrand and PPettit, 2001).

Table 1-9 ABO blood group system (Hoffbrand and PPettit, 2001)

Blood group	**Sub-group**	**Antigens**	**Antibodies**
		On red cells	In plasma

A	A1	A+A1	Anti-B
	A2	A	(Anti-A1)
B	-	B	Anti-A
			(Anti-A1)
AB	A1	A+A1+B	None
	A2B	A+B	(AntiA1)
O	-	(H)	Anti-A
			Anti-Al
			Anti-B
			AntiAlB

Antibody-Al found in 1-2% of A2 subjects and *25*-30% of

cross reacting with both A and B cells. The amount of H antigen is

influenced by the ABO group cells contain most H and A1B cells least

Anti-H may be found in occasional Al and A1B subject (Tolil 1995)**.**

1-2-8 ABO subgroups

A subgroups

In 1911 von Dungern described two different A antigens Based on reactions with A and A1 antisera. Serum from group B individuals contains a mixture of two antibodies, anti A and anti Al, which can be separated by absorption, techniques using appropriate red cell. Group A red cells that react with anti A only and not with Anti Al are classified as A2 subgroup. When anti A purposely absorbed from the serum of group B individual using A2 red cells, the serum left after the cells and attached anti A are removed by centrifugation is referred to as absorbed serum AntibodyA1. Group A red cells that react with both A and A1antibody and are classified as A1 (Hoffbrand and PPettit, 2001)

Table 1-10 A1 versus A2 phenotype (Hoffbrand and PPettit, 2001).

Blood group	Anti A form B seara	Anti A_1 lectin
A_1	+	+
A_2	+	Negative

Another source of Anti-A1 besides absorbed serum is anti-A1 lectin. Lectins are seed extracts that agglutinate human cells with some degree of specificity. The seeds of the plants. DoLichos biflorus serve as the source of the anti A1 lectin. This reagent agglutinates A1 or A1B cells but does not agglutinate A2 or A2B cells. Classification into A1 and A2 phenotypes accounts for 99 percent of all groups A individuals. The cells

of approximately 80 percent of the group A population are A1, and the remaining 20% are A2 weak subgroups. The difference between A1 and A2 is both quantitative and qualitative. and the production of both types of antigens is still a result of an inherited gene at the ABO locus inheritance of an A1 gene converts almost all of the H precursor structure to A1antigens on the red cells because this gene elicits high concentration of the enzyme N-acetylgalactosaminyl transferase , remember this enzyme transfersthe immunodominant sugar N-acetyL galactosamine to the H antigen conferring A specificity to these red cells. A1 is Avery potent gene that creates from 810,000 to 1.1700, 000.A1 antigen sites on the adult A1 red cell .Inheritance of an A2 gene results in the production of only 240.000 to 290.000, A2 antigen sites on the adult A2 red cell this quantitative difference have been reflected not only in the number of antigen sites but also in the concentration of N-acetyl galactos aminyltransferase. **(**Hoffbrand and PPettit,2001).

Studies on the transferases from A1 and A2 individuals have demonstrated greater activity in the sera of A1 individuals than in A2 individuals by their ability to convert group O cells to A cells qualitative differences also exist, inasmuch as 1 to 8 percent of A2 individuals produce anti - A1 in their serum and 22 to *35* percent of A2B individuals produce anti -A1 in facts one investigators have demonstrated that anti Al can be found in the sera of all A2B individuals if sensitive techniques are

used. Therefore some subtle qualitative differences between A1 and A2 antigens must exist even through the same immunodominant sugar are attached by the same transferase in each case. There must be some change in the antigenic structure , because the A2 and A2B individuals cannot recognize the A1 antigen as being part of their own red cell makeup and are immunologically stimulated to produce specific A1 antibody that does not cross react with A2 red cells. .Antibody-A serum from group B donors contains two antibodies antibody A plus antibodyA1 therefore this antibody mixture will react bother A1 and A2 red cells , pure anti A1 antibody will react only with A1 anti gene sites . Regardless of which conceptual presentation is used , the fact remains that group A red cells can be subdivided by the results of tests with anti A From B donor sera Anti- A1B from O donor sera and Anti-A1 from absorbed serum or lectin **(**Hoffbrand and PPettit 2001).

Most group A infants appear to be A2 at birth inasmuch as ABO antigens are not fully developed on the red cells at this time, However, no difficulty is usually encountered grouping cord red cells because most reagents contain potent anti A and anti A1B **(**Hoffbrand and PPettit,2001).

Most cord A2 cell will eventually group as A1 individuals after given amount of time for development usually a few months. Group AB red cells can also be similarly classified into subgroups. To include these

subgroups in the genetic pathways of the biosynthesis of ABH antigens, we must again start with the basic precursor substance **(**Hoffbrand and PPettit,2001).

1-2-9 Sub group B

Sub group B are even rare than sub group A.

B3 this subgroup shows a mixed-field agglutination pattern with anti-B the saliva of secretors of this subgroup possesses B substance.

Bx this subgroup show a weak agglutination pattern with anti-B the saliva of secretor contains a type of B substance (B-like) which inhibits the reaction Between anti-B is found in the serum of individual of this phenotype.

Bei red cell of this phenotype are not agglutination by anti-B but will absorb anti-B, which can subsequently be eluted .H substance but not B substance is found in saliva of secretors (Brecher and Mark, 2005).

1-2-10 Clinical Significance of ABO

ABO antibodies are capable of causing both Hemolytic Disease of the Fetus and Newborn HDFN and Hemolytic Transfusion Reactions HTR. These issues explain the clinical significance of naturally occurring antibodies. HDFN usually presents itself with a maternal antibody of an IgG isotype that corresponds to an antigen on the surface of the baby's red cells. The most common scenario is a group O mother and a group A

baby. ABO hemolytic disease may affect a woman's first pregnancy. This is in contrast to Rh HDFN where the antigenic stimulation usually occurs in the first pregnancy and subsequent antigen-positive newborns are affected. Hemolytic transfusion reaction occurs when a recipient is transfused with red cells that are an ABO group incompatible with the antibodies in his or her serum. Because of the complement-binding ability of the ABO antibodies, this is always a life-threatening situation. As the recipient antibodies react with the incompatible red cells, complement is activated and in vivo hemolysis, agglutination, and red blood cell destruction occurs. ABO compatibility is also significant in solid organ transplantation. For most organs, an ideal scenario for transplant is an ABO compatible solid organ .Post-transfusion antibody titer and aphaeresis to reduce the titer of the incompatible antibody, will assist in achieving a positive outcome when an ABO incompatible organ is transplanted (Jolly JG, 2000).

1-2-11 Gel technology

a gel method to capture haemagglutination, which was developed by

DiaMed AG. A LISS IAT method was devised that eliminated the wash phase and so reduced the potential for error associated with a poor wash technique, as well as contributing to laboratory standardization and economy of effort . The Nowash technique is possible because on

centrifugation, the liquid phase of the reactants remains in the upper chamber of the micro tube and only the cells enter the gel matrix, which contains the AHG. Cells sensitized during the incubation phase react with the AHG and the agglutinates are held at the top of, or throughout, the gel matrix. Non sensitized cells form a pellet at the base of the microtube. Because no liquid enters the gel, there is no danger of neutralization of the AHG, which can happen in a spin-tube method owing to residual human proteins not removed during the wash phase. Weak positive reactions are also more robust in the gel ID-System as there is no possibility of dissociation of the antigen antibody complexes during a too-vigorous wash phase. The sensitivity of antibody detection is, however, still affected by a number of other variables. The serum to cell ratio for a LISS and IAT must be at least 40:1. The period of incubation must be sufficient to allow maximum uptake of antibody and depends on the binding constant of the reaction as well as the concentrations of antibody and antigen. Some weak antibodies may therefore need a longer time to react optimally, or an altered serum to cell ratio (Brecher and Mark, 2005).

1-2-22 Flow cytometry

Although not used as an automated system for routine typing of blood samples, flow cytometry can be applied in special circumstances: for

example, when investigating the cause of double cell populations or for identifying weakly expressed antigens. It can also be used to assess the amount of feto-maternal hemorrhage (FMH) in Rh D negative mothers who require anti-D prophylaxis and for calculating the concentration of anti-D in an immunized RhD negative antenatal woman. The principle of flow cytometry is based on the cells are incubated with antibodies conjugated with fluorophores, dyes that fluoresce under intense light. In the flow cytometer the cells pass, in single file past a laser beam and fluorescence is monitored by photo detectors. As most flow cytometers can detect light of different wavelengths emitted by two or more different fluorophores, more than one antigen can be detected at the same time. Flow cytometer enables large numbers of cells or events to be examined in a short space of time. It is sensitivity is such that a small number of rare events can be detected accurately, if a sufficient number of total events a recounted. This is because the cells arrive at the point of analysis in a random fashion and a subset of cells will also be randomly distributed within the suspension (Brecher and Mark, 2005)

1-3: Rationale

This study can use reference recognition of ABO blood grouping and subgroups for facilitation of safe blood transfusion and possible preparation of Sudanese own panel cells use for antibody screening and antibody identification .

1-4 Objective

General objective

To determination of frequencies of ABO blood grouping and sub group A in Eljaaleen ethnic group.

Specific objectives

-To screen the frequencies of ABO blood grouping in Eljaaleen tribegroup.

- To screen the frequencies of subgroup A in Eljaaleen tribe group.

-To compare the frequency of sub group A phenotypes in Eljaaleen tribe with other ethics groups in Sudan.

Material and Method

2-1 study design

Is a partial descriptive, prospective analytical study conducted in different part of Khartoum state during the period between(May to June), to determine ABO grouping and the subgroup A, among El jaleen tribe.

2-2 Study population

Individuals who related to Eljaleen tribe in Khartoum state.

2-2-1 Inclusion criteria

All individuals who related to Eljaleen tribe in Khartoum state.

2-2-3 Exclusion criteria

Individual who unrelated to Eljaleen tribe in Khartoum state.

2-3 Sampling

Two hundred unrelated blood group A individuals selected randomly from Eljaleen tribe were rolled in this study.

2-4 principle

Antigens on red cells reacted with antibody in the serum resulting in antigen antibody reaction which observed as agglutination (Lewis andBarbra,2001).

2-5 Methodology

Whole blood should be used.

1-glass slide was identified with the patient's or donors name or number

2-A smaller drop (approx 1/4 of the size of the reagent drop) was added from the blood to be tested to antisera (50 µl)

3-mixed well with a clean mixing stick.

4-while rotating the slide, observed macroscopically for agglutination after exactly 20seconds. (Lewis and Barbra,2001)

2-6 Result

a-Strong agglutination positive result

b-No agglutination or reactions between blood and antisera negative (Lewis andBarbra,2001)

2-7 Data analysis

The data of this research ware analyzed by using SPSS computer programmed in which frequency were determined.

Results

200 samples from both sex males 54 % and females 46% table 3-1 with the mean age 23 years .This is analytical cross sectional study was conducted in Khartoum state during the period (January to April 2014) to measurement the frequencies of ABO and A subgroup in Eljaleen tribe in Khartoum state .the results of this study showed that, blood group O was the most predominate 40 % followed by blood group A%34 and B 22% and the less common was AB 4%table 3-2 .The predominate A subgroup was A1 89.71% and other subgroup was 10.29% table 3-3 .

Table 3-1 Distribution of study population according to gender

Gender	Number	Percent
Males	108	54%
Females	92	46%

Male constituted 54% while female were 46% of the total population.

Table 3-2 Frequency and percent of ABO antigen in study population

Group	Frequency	Percent
A	68	34.0%
B	44	22.0%
O	80	40.0%
AB	8	4.0%

The most frequency of ABO blood group was O followed by group A .The least frequent blood group was AB.

Table 3-3 Frequency and percent of A sub group in study population

Group	Frequency	Percent
A1	61	89.71%

Subgroup A1 constituted 61% the remain was other subgroup

Chapter Four

Discussion, Conclusions and recommendation

4-1 Discussion

In this study which was conducted in 2013 to 2014 in Khartoum state to determine the frequencies of ABO blood grouping and A sub grouping among two hundred unrelated individuals from Eljaleen tribe group. The highest incidence was O blood group and AB blood group was lowest incidence table 3-1.The A1 subgroup frequencies in Eljaleen tribe was highest incidence and other subgroup A was lowest incidence table 3-2.The results of this study were compared with the results of other Sudanese ethic groups and were found that were similarity in some ABO blood group antigens and A subgroups with the Al-Shaigia, Al-Danagla and Al-Dinka where the workers reported highest frequency of O blood group and lowest was AB, also A1 was highest frequency and other subgroups was lowest frequency (Fathelrahman ,2010) (Abo Algasim *et.al*2007),also there were differences with the Al-Hassania, Meseria, Al-

Noba, Al-shokria, Al-Arakieen Al-Halaween, Al-Mahs and Al-Hwsa Sudanese ethic groups (Ayaa Abdullah Mahmoud,2010).This study concluded that the similarity between Sudanese tribes that living in one area resulting from interaction and intermarriage between them, while the differences in spectrum may resulted due to sampling error, genetic factors and living in different geographical areas.

4-2 Conclusion

In conclusion ABO and subgroup A phenotypes variation from people to other according to ethnic groups , races diversity associated with genetic concept. This results which obtained from two hundred unrelated individuals males and females concluded that, the frequency of blood group O was the most common than other blood groups in Eljaleen tribe in Khartoum state and A1 was the most common subgroup A (FathelrahmanM.H,2010).

4-3Recommendation

Since the hemolytic transfusion reaction due to A1 or other A subgroups has clinical significance and from this study we can recommended:

-All Sudanese ethnic groups must be tested for detection of blood subgroup particularly A1because it is common in Sudanese population for proper management of pre- transfusion screening test.

-The immune diffusion card method or ID microtyping technique is reliable ,precise, and accurate method for detection of ABO blood groups and A subgroups and should be induced in all national and regional blood banks in Sudan.

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Appendix (I)

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology

Study for determination the frequency of ABO blood grouping and A subgroup in Eljaleen tribe:

-Name:

-Age:

-Sex :

-ABO group:

-Subgroup A:

-Date:

Appendix (II)

كلية الدراسات العليا

برنامج ماجستير مختبرات طبية

علم الدم ومبحث المناعة

إبـراء ذمـة

الاسم :.....

سوف يتم اخذ عينة من الدم الطرفي بواسطة الوخز بإبره طعن وذلك بعد مسح منطقة اخذ العينة بواسطة المطهر . كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها كل وسائل السلامة المعملية.

و انا اقر بان هذه العينات سوف يتم تحليلها لغرض البحث فقط.

أوافق انا المذكور أعلاه علي اخذ عينة لإجراء الدراسة .

الإمضاء : .....

التاريخ: .....