

Chapter one

1. Introduction and literature review

1.1. Introduction

In 1901, Landsteiner demonstrated that human beings could be classified into 4 groups depending on whether their red cells contain one 'A' or another 'B' agglutinogenes or both 'AB' or neither 'O'. It was demonstrated that there are antibodies to A and B. It was also shown that a person's serum does not contain the antibodies for the antigen present in his own red cells but carries antibodies against the antigen which he does not possess (Gupte, 2000).

The second blood group system of great clinical significance was discovered 40 years after Landsteiner's blood groups and it was named Rhesus 'Rh' system. The discovery of Rh and particularly recognition of Rh blocking antibodies attracted new interest in the immunology and genetics of blood groups. In between, 'MN' and 'P' systems were recognized. Now some 100 recognized antigens systems composed of more than 500 different blood groups are known. 'ABO' and 'Rh' systems are the major blood group antigens (Gupte, 2000).

Knowing some information on the distribution of blood group in any population group are useful in population genetic studies and researching population migration patterns, but the most importance of knowing 'ABO' and Rh blood groups phenotypes is in safety of blood transfusion practice and organs transplantation (Bakare *et al*, 2006).

Though there are many studies about frequencies of 'ABO' and 'Rh' groups around the world, published data regarding this issue is rare in Sudan.

This study aimed to provide essential information about the frequency of ‘ABO’ and ‘Rh-D’ blood groups phenotypes in Sudanese blood donors to help in setting of blood bank policy for providing blood products for patients who require blood transfusion.

1.1.1. History of blood group discovery:

Blood group antigens not yet discovered at the start of blood transfusion, which was first carried out between dogs. The first transfusion to human was performed from animal. Blundell (1824) was first who performed human to human blood transfusion, and before that he had established very important points; the first was the dogs which had been bled could be revived by transfusion of dogs blood, and the second was that a transfusion to a dog of even small amount of blood of another species could be fatal; indicating the need to use a donor of the same species. This was fully confirmed by Ponfick (1875) who showed that if red cells of a donor from another species were transfused they underwent rapid intravascular lysis (Millson *et al*, 1993).

1.1.2. Red cell antigens:

In humans, 26 blood group systems with 228 antigens have been identified (table 1-1). Additional antigens have been identified but have not been assigned to established systems. Red blood cell antigens may be proteins, glycoproteins, or glycolipids. Most red cell antigens are synthesized by the red cells; however, some antigens, such as those of the Lewis and Chido/Rogers systems, are adsorbed onto the red cell membrane from the plasma. Some red cell antigens are specific to red cells; however, others are found on other cells throughout the body (John *et al*, 2003).

Each system is a series of red cell antigens, determined either by a single genetics locus or very closely linked loci. Apart from those of the ‘ABO’ system, most of these antigens were detected by antibodies stimulated by transfusion or pregnancy (Lewis *et al*, 2006).

Alternative form of gene coding for red cell antigens at a particular locus are called alleles, and individuals may inherit identical or non-identical alleles. Most blood

group genes have been assigned to specific chromosomes (e.g. 'ABO' system on chromosome 9, Rh system on chromosome 1). The term genotype is used for the sum of the inherited alleles of particular gene (e.g. AA, AO), and most red cell genes are expressed as co-dominant antigens (i.e. both genes are expressed in the heterozygote).

The phenotype refers to the recognizable product of the alleles, and there are many racial differences in frequencies of red cell phenotypes (Lewis *et al*, 2006).

The main clinical importance of a blood group system depends on the capacity of the alloantibodies (directed against the antigens not possessed by the individual) to cause destruction of transfused red cells or to cross the placenta and give rise to hemolytic disease in fetus or newborn. This in turn depends on the frequency of the antigens and the alloantibodies and the characteristics of the latter-thermal range, immunoglobulin class, and ability to fix complement. On these criteria, the 'ABO' and 'Rh' systems are of major clinical importance. Anti-A and anti-B are naturally occurring and are capable of causing severe intravascular hemolysis after an incompatible transfusion. The 'Rh-D' antigen is the most immunogenic red cell antigen after 'A' and 'B', being capable of stimulating anti-D production after transfusion or pregnancy in majority of 'Rh-D' negative individuals (Lewis *et al*, 2006).

Table 1-1: Blood group systems recognized by the ISBT working party(Lewis *et al*, 2006)

System number	System name conventional	System symbol ISBT	Gene(s)
001	ABO	ABO	ABO
002	MNS	MNS	GYPA GYPB
003	P	PI	P
004	Rh	RH	RHD RHCE
005	Lutheran	LU	LU
006	Kell	KELL	KEL
007	Lewis	LE	FUT3
008	Duffy	FY	FY
009	Kidd	JK	HUT11
010	Diego	DI	SLC4A1
011	Yt	YT	ACHE
012	Xg	XG	XG
013	Scianna	SC	SC
014	Dombrock	DO	DO
015	Colton	CO	AQP1
016	LW	LW	LW
017	Chido/Rogers	CH/RG	C4A,C4B
018	H	H	FUT1
019	Kx	XK	XK
020	Gerbich	GE	GYPE
021	Cromer	CROM	DAF
022	Knops	KN	CR1
023	Indian	IN	CD44
024	Ok	OK	OK
025	MER2	RAPH	MER2

*ISBT, International Society of Blood Transfusion.

1.1.3. ABO System:

Discovery of the 'ABO' system by Landsteiner marked the beginning of safe blood transfusion. The 'ABO' antigens, although most important 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 transplants (e.g. group 'A' stem cell into a group 'O' recipient) will provoke hemolysis, unless the donation is depleted of red cells (Lewis *et al*, 2006).

1.1.4. ABO antigens and encoding genes:

The theory of the inheritance of the 'ABO' blood group was first described by Bernstein in 1924. 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 (Mehdi, 2006).

There are four main blood groups: 'A', 'B', 'AB', and 'O'. The epitopes of 'ABO' antigens are determined by carbohydrates (sugar), which are linked either to polypeptides (forming glycoproteins) or to lipids (glycolipids). The expression of 'ABO' antigens is controlled by three separate genetic loci: 'ABO' located on chromosome 9 and 'H' and 'Se', both of which are located on chromosome 19. Each gene codes for different enzyme (glycosyl transferase), which attaches specific monosaccharides onto precursor disaccharide chains. There are four types of disaccharide chains, the Type 1 is found in plasma and secretion and is the substrate for the 'Se' gene, whereas Type 2, 3, and 4 chains are only found on red cells and are the substrate for the 'H' gene. It is likely that the 'O' and 'B' genes arose by mutation of the 'A' gene. The 'O' dose not encode for the production of a functional enzyme.

The expression of 'A' and 'B' antigens is determined by 'H' and 'Se' genes, which both give rise to glycosyl transferases that add L-fucose, producing the 'H' antigen.

The presence of ‘A’ or ‘B’ gene (or both) results in the production of further glycosyltransferases, which convert ‘H’ substance into ‘A’ and ‘B’ antigens by the terminal addition of N-acetyl-D-galactosamine and D-galactose respectively. Because the ‘O’ gene products an inactive transferase, ‘H’ substance persists unchanged as group ‘O’ (table 1-2) (Lewis *et al*, 2006).

The ‘H’ antigen content of red cells depends on the ‘ABO’ group and when assessed by agglutination reactions with anti-H, the strength of reaction tend to be graded O>A2>A2B>B>A1>A1B (Lewis *et al*, 2006).

The ‘A’, ‘B’ and ‘H’ antigens are detected early in fetal life but are not fully developed on the red cells at birth. The numbers of antigen sites reach “adult” level at around 1 year of age and remain constant until old age, when a slight reduction may occur (Lewis *et al*, 2006).

Table 1-2: ABO Genes and Antigens (John *et al*, 2003).

Phenotype	Antibody	Antigen	Gene Product
A	Anti-B	N-acetylgalactosamine	A transferase
B	Anti-A	D-galactose	B transferase
AB	None	N-acetylgalactosamine and D-galactose	A transferase and B transferase
O	Anti-A and anti-B	L-fucose	Absent or nonfunctional A or B transferase
A2	Anti-B and variable amount of anti-A 1	N-acetylgalactosamine	A transferase

1.1.5. Bombay blood group

The 'O' blood group individuals normally do not carry either 'A' or 'B' antigen but maximum amount of 'H' antigen. Some individuals lack even 'H' substance along with 'A' and 'B'. These individuals are 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 other antibodies found in any 'O' blood group. This anti-H of Oh is clinically significant, warm antibody reactive at 37°C (Mehdi, 2006).

There is other null phenotype in the 'ABO' system called Para Bombay, in which an absence of only a trace of 'ABH' antigens is detected on the red cells. Both Bombay and Para Bombay individuals possess mutant H gene (hh) (Hofbrand and Pettit, 2001).

This blood group was first discovered in Bombay, hence its name, by Bhende et al in the year 1952. The Bombay blood group is not compatible with any 'O' blood group, and the only choice of blood for these individuals remains only Bombay itself (Mehdi, 2006).

1.1.6. ABO subgroups:

The subgroups of 'A' and 'AB' are of clinical significance.

1.1.6.1. Subgroups of A:

The group 'A' has been divided in 'A1' and 'A2' depending on their reaction to antisera anti-A and anti-A1.

A1: the 'A' red cells which react with both anti-A and anti-A1 is designated as 'A1' subgroup. This has more antigenic sites for 'A' and less of 'H'. The antibody present is only anti-B (Mehdi, 2006).

A2: the 'A' red cells which react with anti-A only and not with anti-A1 is called 'A2'. This is a weak 'A' group and it carries more 'H' substance. In 1-8% of cases of 'A2' group anti-A1 is also present beside anti-B (Mehdi, 2006).

The cells of approximately 80% of 'A' individuals is 'A1' while the remaining 20% are 'A2'. There are a few weak 'A' subgroups to which are designated as 'A3', 'Ax' and 'Am' (Mehdi, 2006).

1.1.6.2. Subgroups of AB:

Like 'A' the 'AB' is also divided in 'A1B' and 'A2B' subgroups. The 'A1B' cells carry minimum amount of 'H' antigen. Approximately 22-35% of 'A2B' individuals produce anti-A1. The anti-A1 present in 'A2' or 'A2B' individuals is usually a cold reactive clinically insignificant antibody, unless it reacts at 37°C (Mehdi, 2006).

1.1.7. Secretors and non-secretors:

The secretor locus has been assigned to chromosome 10-19 and is linked with the 'H' and 'Lu' (Lutheran) loci. The alleles on the secretor locus are 'Se' and 'se'. about 80% of random population are secretors (genotype SeSe or Sese) and 20% are nonsecretors (genotype sese) (Gupte, 2000).

Secretors have 'H' substance in the saliva and other body fluids together with 'A' substance, 'B' substance, or both depending on their blood group. Only traces of these substances are present in the secretion of nonsecretors, although the antigens are expressed normally on their red cells and other tissues. An individual's secretor status can determine by testing for 'ABH' substance in saliva (Lewis *et al*, 2006).

1.1.8. ABO antibodies:

'ABO' antibodies, in the absence of the corresponding antigens, appear during the first few months after birth, probably as a result of exposure to 'ABH' antigen-like substances in the diet or the environment (i.e. they are "naturally occurring").

The antibodies are a potential cause of dangerous hemolytic transfusion reactions if transfusion is given without regard to 'ABO' compatibility (Lewis *et al*, 2006).

1.1.8.1. Anti-A and Anti-B:

Anti-A and anti-B are always, to some extent, immunoglobulin M (IgM). Although they react best at low temperature, they are nevertheless potentially lytic at 37°C. hyperimmune anti-A and anti-B are predominantly of IgG class and are usually produced by group 'O' and sometimes by group 'A₂' individuals. Hyperimmune IgG anti-A and/or anti-B from group 'O' or group 'A₂' mothers may cross the placenta and cause HDN (Lewis *et al*, 2006).

1.1.8.2. Anti-A₁ and Anti-H:

Anti-A₁ reacts only with 'A₁' and 'A₁B' cells and is occasionally found in the serum of group 'A₂' individuals (1-8%) and A₂B (25-50%). However, anti-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 (Lewis *et al*, 2006). The anti-A₁ can also be extracted from lectin seed called *Dolichos biflorus* (Gupte, 2000).

Anti-H reacts most strongly with group 'O' and 'A₂' red cells and also normally act as a cold agglutinin. 'A' notable, but rare exception is the anti-H that occurs in the Oh Bombay phenotype, which is an IgM antibody and causes lysis at 37°C so that Oh Bombay phenotype blood would be required for transfusion (Lewis *et al*, 2006).

1.1.9. History of Rh system discovery:

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. It was reported by Levine and Stetson in the year 1939 (Mehdi, 2006).

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

agglutinated the red cells of 85% of the human beings tested. The antibody was called Anti-Rh and its antigenic determinant 'Rh' factor (Mehdi, 2006).

Wiener and Peters, also in the year 1940 isolated human anti-Rh antibody from the sera of individuals transfused with 'ABO' compatible 'Rh+' 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 (Mehdi, 2006).

1.1.9.1. Rh system:

The 'Rh' system is only next in importance to 'ABO' system in transfusion practice. The importance of this system lies in the high immunogenicity of 'Rh-D' antigen, which readily induces formation of anti-D antibodies in 'Rh-D' negative individuals. Anti-D antibodies can cause hemolytic transfusion reaction or, in pregnant women, 'Rh' hemolytic disease of newborn (Kawthalkar, 2013)

Rh antigens are well developed before birth and can be demonstrated on the red cells of very early fetus (Lewis *et al*, 2006).

1.1.9.2. Rh antigens and encoding genes:

The five commonly detected antigens are 'D', 'C', 'E', 'c' and 'e', of which 'D' is the most potent and highly immunogenic, followed by 'c' and 'E'. The commonly used term of 'Rh+' and 'Rh-' depends on the presence or absence of 'D' antigen (Mehdi, 2006).

There are different genetic theories of 'Rh' system, which are:

1.1.9.1.1. a. Fisher and Race theory: which proposed that the production of these 'Rh' antigens was controlled by 3 sets of alleles whose loci were so closely linked that crossing over between them hardly occurred. These 3 loci were 'D' and 'd', 'C' and 'c', 'E' and 'e' (Gupte, 2000).

1.1.9.1.2. b. Alexander Wiener et al theory: which proposed that a single gene at 'Rh' locus was responsible for the production of 'Rh' antigens. Wiener's two original genes were designated as 'Rh' and 'rh' to describe those genes that produced Rh₀ 'D' and those that failed to produce Rh₀ 'D', respectively. Wiener's single gene theory gives Wiener's concept of 8 common 'Rh' genes (i.e. r, r', r'', r^y, R⁰, R¹, R², R^Z (Gupte, 2000).

1.1.10. D^u phenotype:

It has been observed that certain 'D' positive red cells are not agglutinated by all anti-'D' sera, but require 'AHG' (Coomb's) sera and ICT to show agglutination. This phenomenon is nothing but a weak expression of the 'D' antigen. This particular 'D' phenotype is called 'D^u'. So 'D^u' is not different antigen but different expression of 'D' antigen (Mehdi, 2006).

There is a quantitative reduction in the number of 'D' antigen sites on such red cells. 'D^u' recipients do not make anti-D antibodies following stimulation by 'D' antigen. 'D^u' donors units are considered 'Rh' positive and transfused only to 'Rh' positive recipients (Kawthalkar, 2013).

If the mother carries anti-D antibody then the 'D^u' infant is likely to suffer from HDN (Mehdi, 2006).

1.1.11. Partial D antigen:

In red cells having partial 'D' antigen, parts of 'D' antigen are missing. Variants of partial 'D' antigen exist. Individuals with 'D^{VI}' variant are able to produce anti-D antibody against the missing part of the antigen if exposed to 'D+ve' antigen. Such recipients should be considered as 'Rh' negative, while donors should be regarded as 'Rh' positive (Kawthalkar, 2013).

1.1.12. Rh null phenotype:

Complete absence of 'Rh' antigens may be associated with a congenital hemolytic anemia with spherocytes and stomatocytes in the blood film, increased osmotic fragility, and increased cation transport (Lewis *et al*, 2006).

The presence of the 'Rh' proteins in the red cell membrane appears to be necessary for the expression of other membrane proteins such as the 'LW', 'Duffy', and 'U' antigens. 'Rh' null cells have been demonstrated to lack the 'LW' and 'Fy5' antigens and have weakened expression of the 'S', 's', and 'U' antigens. Fortunately, the 'Rh' null phenotype is rare, as these individuals form an alloantibody (anti-Rh29) that reacts with all other red cells except 'Rh' null when they are transfused. Thus, obtaining compatible blood can be challenging (John *et al*, 2003).

1.1.13. Rh antibodies:

Except for anti-C and anti-E that occur without known stimulus (Mehdi, 2006), in general, most 'Rh' antibodies are of immune type, i.e. they are the result of immunization by blood transfusion or pregnancy. Most of these antibodies are of IgG class. In practice, 'Rh' antibodies can cause HTR or HDN. Since 'Rh' antibodies do not activate complement, hemolysis is extravascular and predominantly occurs in spleen. Due to high immunogenicity of D antigen, Rh negative persons (especially women of child bearing age) should be transfused only with 'Rh' negative blood. During pregnancy, IgG anti-D can cross the placenta and induce HDN by causing immune hemolysis of fetal red cells. 'Rh' HDN can be prevented by prophylactic administration of 'Rh' immune globulin to all 'Rh-ve' women during mid-pregnancy and within 72 hours of delivery. Anti-D and anti-c can cause severe HDN. Anti-C, anti-E, and anti-e usually do not cause HDN or cause mild HDN (Kawthalkar, 2013).

1.1.14. Rh nomenclature:

1.1.14.1. Rh-Hr Terminology:

Wiener proposed that gene product is a single entity agglutinin on the surface of red cells and each agglutinin has number of antigens, recognized by its own specific antibody.

A capital R denotes the presence of the original factor in an agglutinin, whereas r as subscript indicates the lack of the factor. Rh₀ represents 'D' while rh' and rh'' represent 'C' and 'E' (Mehdi, 2006).

1.1.14.2. DCE Terminology:

Fisher and Race in 1944 defined the antigens of the 'Rh' system as 'D', 'C', 'E', 'c' and 'e'. the same letters were used for gene too, but the gene were written in italics. The corresponding antibodies of these antigens are anti-D, anti-C, anti-E, anti-c and anti-e. This is the easiest and widely accepted system of nomenclature (Mehdi, 2006).

1.1.14.3. Numerical Terminology:

Rosenfield and coworkers in 1960 proposed this system which assigns a number to each antigen. The corresponding names of the systems of Fisher and Rosenfield are as follows:

- 'D' is Rh1
- 'C' is Rh2
- 'E' is Rh3
- 'c' is Rh4
- 'e' is Rh5

The absence of an antigen is designated by a prefix negative sign, e.g.:if c and e antigens are absent, the designation is: Rh: 1, 2, 3, -4,-5 (Mehdi, 2006).

Table 1-3: Fisher Race and the corresponding short notations: (Lewis *et al*, 2006)

Fisher	Short Notations
CDe	R ¹
cDE	R ²
cDe	R ⁰
CDE	R ^z
Cde	r'
Cde	R
cdE	r''
CdE	r ^y

1.1.15. Clinical significance of Rh system:

Clinical complication result from RBCs destruction due to the interaction of an alloantibody with RBCs carrying the corresponding antigen. The 'Rh-D' antigen is highly immunogenic and induces an immune response in 80% of 'Rh-D' negative persons when transfused with 200 ml of 'D-positive' blood (Millson *et al*, 1997).

1.1.16. Rhesus and hemolytic disease of newborn (HDN):

Alloimmunization against the 'Rh-D' antigen during pregnancy is the most frequent cause of HDN. Immunization occurs when fetal red cells carrying antigen inherited from the father enter the mother's circulation following fetomaternal bleeding, when the mother does not express the same antigen may produce IgG antibodies towards the fetal antigen and these antibodies can pass through the placenta causing a diversity of symptoms ranging from mild anemia to death of the fetus (Avant and Reid, 2000).

1.1.17. Blood transfusion:

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and blood products (Reid, 2008).

Much of the routine work of a blood bank involves testing blood form both donors and recipients to ensure that every individual is giving blood that is compatible and as safe as possible. If a unit of incompatible blood is transfused to an individual, severe acute hemolysis reaction, renal failure and shock is likely to occur and death is possible. Antibodies can be highly active and can attack RBCs and bind components of the complement system to cause massive hemolysis of the transfused blood (Reid, 2008).

Patients should ideally receive their own blood or type-specific blood products to minimize the chance of transfusion reactions. Risks can be further reduced by cross-matching blood, but this may be skipped when blood is required for an emergency. Cross-matching involves mixing a sample from recipient's serum with a sample from donor's red cells and checking if the mixture agglutinates, or forms clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination with a microscope. If agglutination occurs, that particular donor's blood cannot be transfused to that particular recipient. In a blood bank it is vital that all blood specimens are correctly identified, so labeling has been standardized using a barcode system known as ISBT 128 (Reid, 2008).

Table 1-4: Comparison of ABO and RhD groups: (Kawthalkar, 2013)

Parameter	ABO group	RhD group
1. Location of gene	Chromosome 9	Chromosome 1
2. Antigens	A, B, AB	D
3. Distribution of antigens	Red cell, platelets, many tissues, body fluids	Red cell only
4. Development of antigens	Weak expression at birth	Fully developed at birth
5. Dosage effect*	No	Present
6. Nature of antibodies	Naturally occurring	Immune
7. Antibody class	IgM	IgG
8. Whether antibodies fix complement	Yes	No
9. Optimal reaction temperature of antibody	4°C	37°C
10. Optimal reaction medium	Saline	Antihuman globulin

*Dosage effect: A situation where antibody reacts more strongly with red cells having double the dose of antigen (due to homozygous state) than those having single dose (heterozygous state).

1.2. Previous studies:

There are many studies about ‘ABO’ and ‘Rh-D’ frequencies around the world.

A total of 22897 subjects in District Swat, Pakistan were grouped for ‘ABO’ and ‘Rh-D’. (74.86%) were male subjects and (25.140%) were female. (90.99%) of male subjects and (87.56%) female subjects were found to be Rh-positive. The frequency of Rh-negative group in male subjects were (9.01%) where as in female subjects were (12.22%). The frequency of ‘A’, ‘B’, ‘O’ and ‘AB’ groups in Rh-positive male

subjects were (25.63%), (29.54%), (26.04%) and (9.78%), amongst female subjects, it was (24.53%), (28.06%), (25.54%) and (9.43%) respectively. In Rh-negative male subjects the frequency of 'A', 'B', 'O' and 'AB' is (2.25%), (2.88%), (3.01%) and (0.88%), while amongst females it is (3.54%), (4.24%), (3.74%) and (0.92%) respectively (Khattak, 2008).

The distribution of 'ABO' and 'Rh-D' blood groups among 150,536 blood donors in a tertiary care center in South India was studied over a period of 11 years (April 1988 to March 1999). The most common blood group was found to be group 'O' (38.75%), followed by group 'B' (32.69%), and group 'A' (18.85%). The least common blood group was 'AB' group (5.27%). 'A2' or 'A2B' groups were found in 3.01% and 1.43% of donors, respectively. The prevalence of 'Rh-D' negative group was found in (5.47%) donors. Bombay group (H negative non-secretor, genotype hh phenotype Oh) was found in 6 donors (0.004%). Although the incidence of 'Rh-D' negative group was identical to previously published data from North India, the most common blood group was 'O' group in our study as opposed to 'B' group (Das *et al*, 2001).

A study from a tertiary care teaching hospital of Kumaon region of Uttarakhand on prevalence of 'ABO' and 'Rh' blood groups in blood donors is reported. The most common blood group was 'B' (32.07%) and least common being 'AB' (10.53%). Blood group 'O' and 'A' had same frequency. The prevalence of 'Rh' positive and negative distribution in the studied population was 94.49% and 5.51% respectively. Male donors were more than female donors, ratio being 352:1(0.3%). Blood group frequency with respect to 'ABO' and 'Rh' positive was found to be shown by formula B> O>A >AB. The frequency for 'ABO' and 'Rh' negative was given by the formula B>A>O>AB (Garg *et al*, 2014).

Over a twenty-year period between 1986 and 2005, a total of 160,431 blood samples were grouped for 'ABO' and 'Rh-D' at the blood bank of the University of Benin Teaching Hospital, Benin City, Nigeria. Blood group distribution among these

samples showed phenotypes as 'A'=23.72%, 'B'=20.09%, 'AB'=2.97%, and 'O'=53.22%. The 'Rh-D' negative phenotype was found among 6.01% of the samples tested (Enosolease and Bazuaye, 2008).

A study on Frequency of 'ABO' blood groups in the Eastern region of Saudi Arabia was conducted. The study included a total of 57396 male potential blood donors; 19496 blood donors between the years 1985-1989 (referred to as first period of study) and 37700 blood donors between the years 1995-1999.

Overall frequency of 'ABO' and 'Rh' blood groups during the first and second periods were the following: 'O' positive 48% and 46%; 'A' positive 24% and 24.5%; B-positive, 17% and 17%; AB positive 4% and 4%; O negative 4% and 5%; A negative 2% and 2%, 'B' negative 1% and 2%; and 'AB' negative, 0.23% and 0.32% (Bashwari *et al*,2001).

In 1989 the distribution of the 'ABO' and 'Rh-D' blood groups amongst 3087 random blood donors in Khartoum is reported. Gene frequencies are $ABO*O = 0.6683$, $ABO*A = 0.1914$, $ABO*B = 0.1403$, $RH*D = 0.7436$. The figures are similar to those last reported 35 years ago which were based on much smaller samples (Khalil *et al*,1989).

1.2.1 ABO and Rh frequencies in Sudan:

Several studies have been carried out to determine the frequency of 'ABO' and 'Rh' blood group antigens and phenotype among the Sudanese ethnic groups.

In a study done in Elmanaseer tribe, the 'O' antigen was the most common antigen among the study group, followed by 'B', 'A' and the least antigen was 'AB' antigen. 'e', 'c', and 'D' antigens were the most frequent in the study group, while 'C', and 'E' antigens were reported as lower frequencies. The 'D^u' antigen was very rare (Amged, 2007).

Similar study in Elshokria tribe to determine the frequency of 'ABO' and 'Rh' antigen and phenotype and it was observed that the most common antigen was 'O' antigen (58%) followed by 'A' antigen (24%), 'B' (14%) and 'AB' (4%).

The percentage of positive-negative 'Rh' antigen was as (96%) positive and (4%) negative (Nahid, 2007).

Another study in Alshigia tribe revealed that group 'O' is the most common (49%), followed by group 'A' (26%), 'B' (22%), and 'AB' was the lowest (3%). The frequency of 'Rh-D' antigen shows that (96%) were positive and (4%) were negative (Sara, 2007).

In another study in Albderia tribe to determine the frequency of 'ABO' and 'Rh' system, the 'O' blood group was the most common group (51%), 'A' (16%), 'B' (32%) and 'AB' (4%). The 'Rh-D' was (96%), 'E' (18%), 'C' (66%), 'e' (91%) and 'c' was (79%) (Elmoeiz, 2007).

Similar study in Alarakeen ethnic group, 'O' antigen was the common phenotype (40%), followed by 'A' (30%), 'B' (26%), 'AB' (4%) and 'Rh-D' (91%) (Malik *et al*, 2007).

1.3. Rationale:

The knowledge of RBC antigen phenotype frequencies in a population is helpful in creating a donor data bank for the preparation of indigenous cell panels and for providing antigen-negative compatible blood to patients with multiple alloantibodies. Moreover, 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 parent identification and in the compatibility investigations before organ transplantation.

To the best of our knowledge, there are no published data on frequency of 'ABO' and 'Rh-D' blood group in most of blood banks in Sudan.

This research will provide an essential data about blood donors for scientific and proper protocol to be used in blood transfusion practice, and also will provide a bigger picture about 'ABO' and 'Rh-D' phenotype in the Central Blood Bank which serves mixture of Sudanese population.

1.4. Objectives:

1.4.1. General objective:

To determine the frequency of 'ABO' and 'Rh-D' blood group antigens and phenotypes among Sudanese blood donors in Khartoum State.

1.4.2. Specific objectives:

1. To determine the 'ABO' frequencies among Sudanese donors.
2. To determine the 'Rh-D' phenotype frequency among Sudanese donors.
3. To compare the 'ABO' and 'Rh-D' phenotype frequencies with sociodemographic characteristics (age and gender) of the studied group and other studies.

Chapter Two

2. Material and methods

2.1. Study design:

This is a descriptive, cross sectional study.

2.2. Study setting:

This study was conducted in Central Blood Bank of Khartoum State between October and November 2015, to determine the ‘ABO’ and ‘Rh-D’ phenotypes frequency among Sudanese donors.

2.3. Study population:

The samples were collected from Sudanese blood donors attended the blood bank during the study period.

2.3.1. Inclusion criteria:

Male or female Sudanese blood donors with aged ranged 18-50 years who donated blood during the study period.

2.3.2. Exclusion criteria:

Non Sudanese donors and donors aged more than 50 years.

2.4. Sample size and sampling technique:

Blood samples from a total of 500 donors were obtained. The technique used in sample collection was non-probability sampling; all donors who came to donate blood in two days a week were involved.

2.6. Methods:

2.6.1. Collection of blood sample:

About 3 ml were obtained from each (CPDA-1) blood bag of 500 blood donors.

2.6.2. Manual method used:

2.6.2.1. ABO grouping:

A set of 'ABO', 'Rh-D' monoclonal IgM antisera (anti-A, anti-B, anti-AB. (Fortress diagnostics) was used and checked for their quality using known (A cell, B cell, O cell) panel cells.

2.6.2.1.1. Principle of ABO grouping:

A test cell (5% suspension or whole blood) is reacted with anti-A and anti-B antisera, and agglutination reaction with certain antisera will indicate the presence of corresponding antigen. Negative reaction indicates the absence of corresponding antigen (Dacie *et al*, 2001).

2.6.2.1.2. Test procedure:

1. Clean dry glass slides were labeled A, B, AB for anti-A, anti-B, and anti-AB antisera respectively.
2. One drop of whole blood was added to the labeled slides.
3. One drop of anti-A, anti-B and anti-AB were added to the blood drops according to the label.
4. The blood and antisera were mixed well and the reaction was read (Mehdi, 2006).

2.6.2.1.3. Interpretation of results:

- Positive reaction is indicated by clumping of cells due to presence of the 'A' or 'B' antigens (agglutination).
- Negative reaction is indicated by absence of clumping and the cells appear free and the preparation was homogenous due to absence of 'A' or 'B' antigens (no agglutination) (Mehdi, 2006).

2.6.2.2. Determination of Rh-D phenotype:

2.6.2.2.1. Test procedure:

1. One drop of anti-D reagent was added to the slide labeled test.
2. One drop of whole blood was added to the drop of reagent.
3. The cell and the reagent were mixed and separate the mixture.
4. The slide was rocked gently for 2 minutes.
5. The results were reported. (Mehdi, 2006).

2.6.2.2.2. Interpretation of results:

Agglutination on the test slide is positive test, and no agglutination on the slide is a negative test.

2.6.2.2.3. Test for D^u:

All 'Rh-D' negative samples by routine 'Rh' grouping were confirmed by D^u method using indirect antiglobulin test (IAT).

2.6.2.2.4. Principle of antiglobulin test:

The incomplete antibodies are not capable of agglutinating the red cells on their own, since a gap remains between to sensitized cells.

The Fab portion of the incomplete antibody (IgG) binds with the antigen on red cells. When AHG serum is added the two Fab portions of the molecule (anti-IgG) attached

to the Fc portions of IgG antibodies already attached to the red cells and so bridging the gap between sensitized cells causing agglutination (Mehdi, 2006).

2.6.2.2.5. Procedure:

1. One drop of anti D was added to the tube labeled test.
2. One drop of whole blood was added to the test tube.
3. Mixed properly and incubated at 37°C for 30 minutes.
4. Centrifuged at 1000 rpm for 1 minute.
5. Dispersed the cell button and examined for agglutination, if shown strong reaction the cells were 'Rh-D' positive.
6. If no agglutination was seen the test sample, the cells washed 3-4 times with saline and discarded the supernatant.
7. Two drops of coomb's reagent was added, mixed properly and centrifuged at 1000 rpm for 1 minute.
8. Dispersed the cell button and examined for agglutination.
9. The results recorded: if the test sample shows agglutination the test is positive for D^u.
10. If the test sample was negative IgG sensitized cells were added as control, the test would show agglutination. It simply confirms the test result and the validity of the procedure (Mehdi, 2006).

2.6.3. Quality control measures:

It was remembered that most of errors in transfusion practice caused by identification mistakes. Manufacture instructions were strictly followed, In addition:

- Positive and negative controls were included. For 'ABO' grouping the red cells were tested with anti-A and anti-B.

- 'Rh-D' grouping was performed using potent anti-D reagent. The results were recorded as 'Rh-D' positive if the test was positive. If the test is negative it was repeated using D^u method. If it still negative the result as recorded negative

2.7. Data analysis:

Data were analyzed by using Statistical Package of Social Science (SPSS) program, descriptive statistics (frequencies and chi-square tests).

2.8. Ethical considerations:

An approval for the work was taken from Research Ethical Committee in Sudan University of Science and Technology and permission from the head of Central Blood Bank was taken.

Chapter Three

3. Results

This was descriptive, cross sectional study carried out during October to November 2015 for determination of 'ABO' and 'Rh-D' frequencies among Sudanese blood donors in the Central Blood Bank, Khartoum, Sudan.

Five hundred separated blood samples were collected from five hundred (CPDA-1) blood bags of donors with ages range between 18-50 years from both genders.

Male donors were (97%) of total study subjects, while female donors were (3%) (Figure 3-1).

The donors were classified according to their age into 4 groups; group one (less than 20 years), group two (20-29 years), group tree (30-39 years), and group four (40-50 years).

Group three were the most frequent (48%) followed by group two (39.2%), then group one (8%) and the fewest donors were from group four (4.8%) (Figure 3-2).

The 'O' antigen was the most common antigen among the study group (45.4%) followed by 'A' antigen (29%), 'B' antigen (20.8%), and the least common antigen was 'AB' (4.8%) (Figure 3-3).

'Rh-D' antigens was present in (92.2%) of group under study, while (7.8%) were negative (figure 3-4).

Statistically There is no significant relationship between blood group and gender (p. value = 0.85), or blood group and age (p. value= 0.94) in this study.

Figure (3-5) shows each blood group with their positive and negative 'Rh-D' frequency.

Figure (3-6) shows each age group and their 'ABO' frequency.

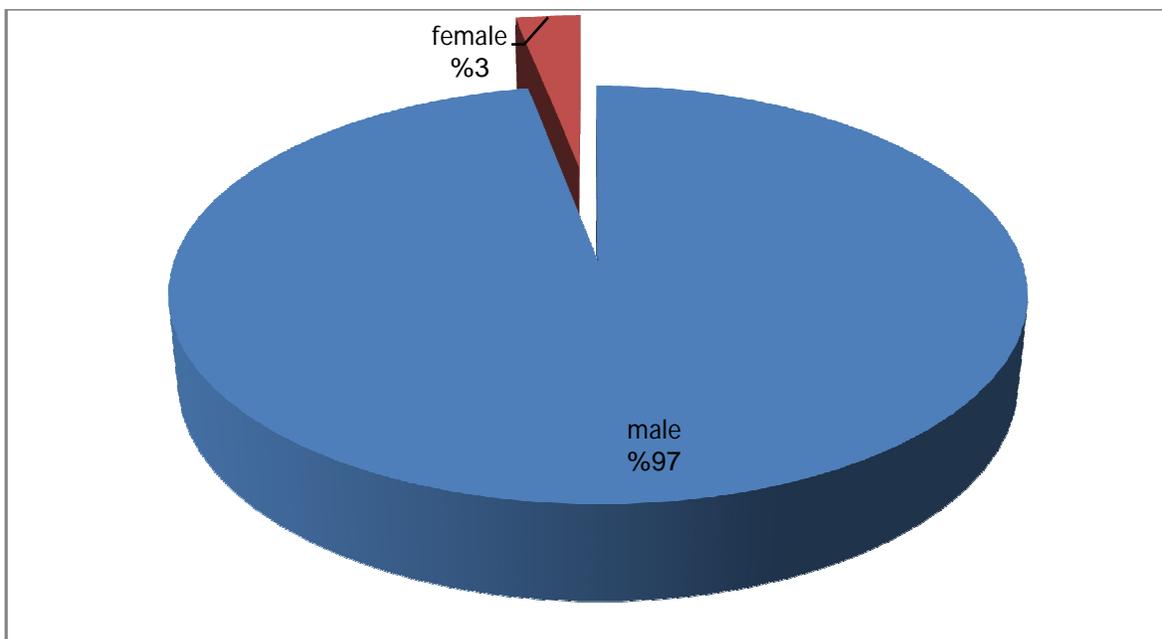


Figure 3-1: Characteristics of the studied population according to gender.

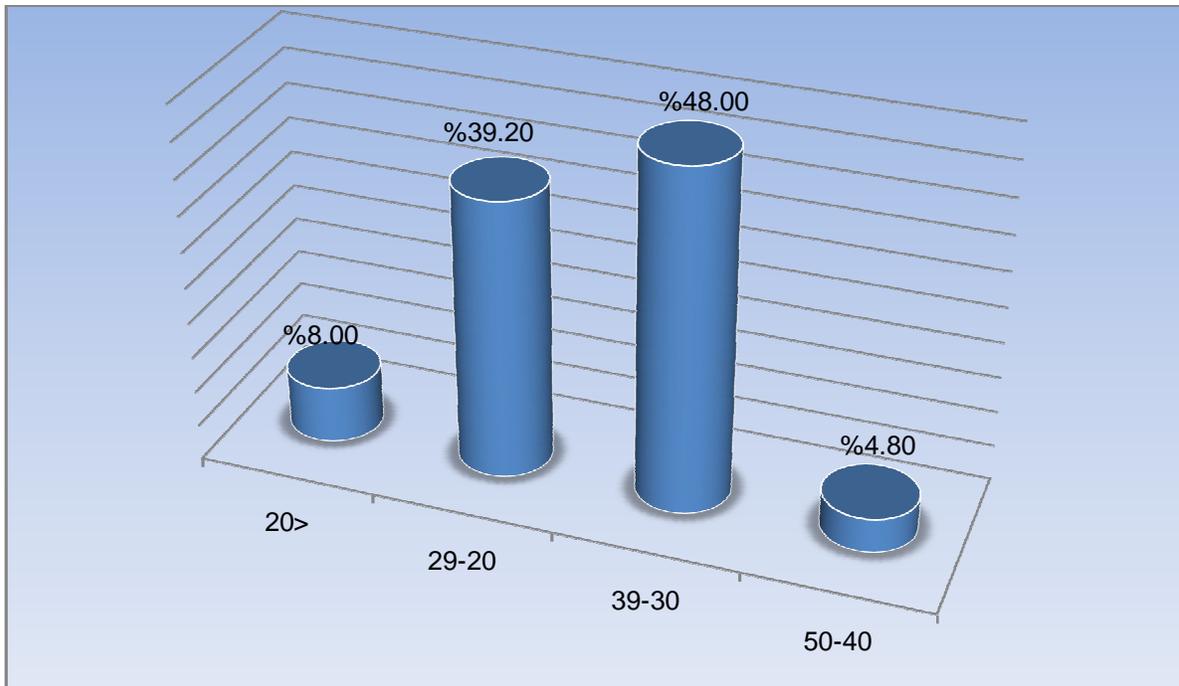


Figure 3-2: Age group frequency among the studied group.

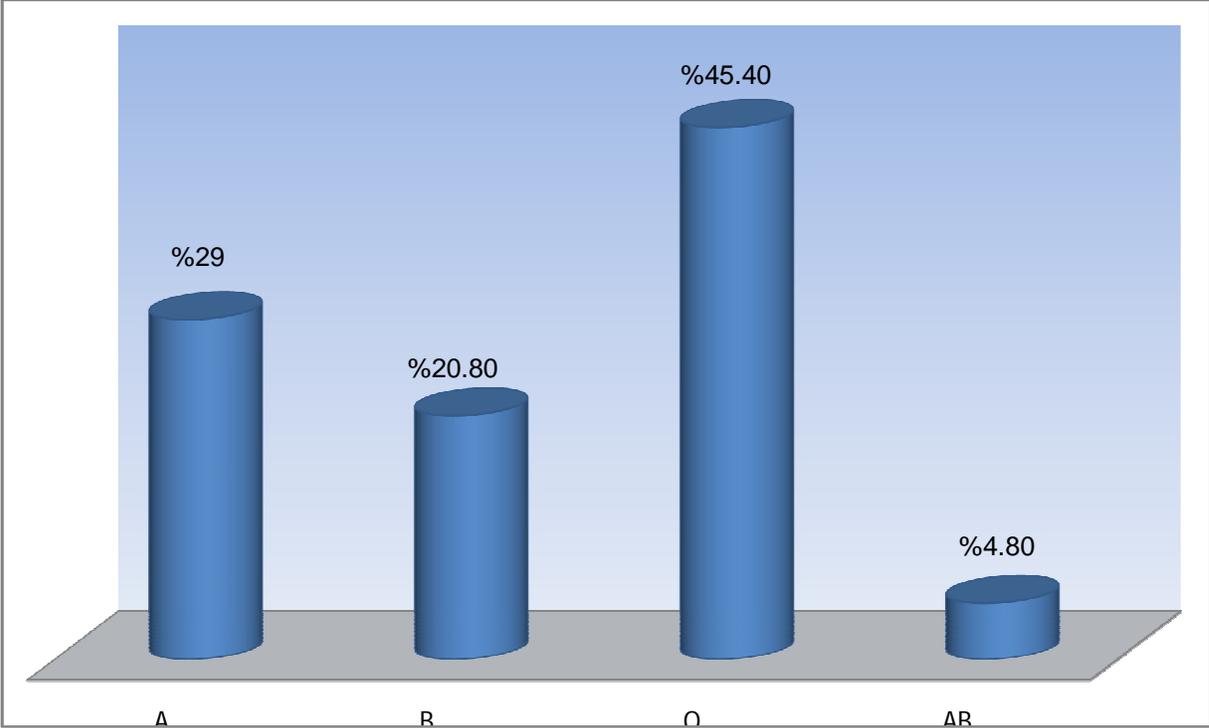


Figure 3-3: Frequency of ABO blood group among the studied group.

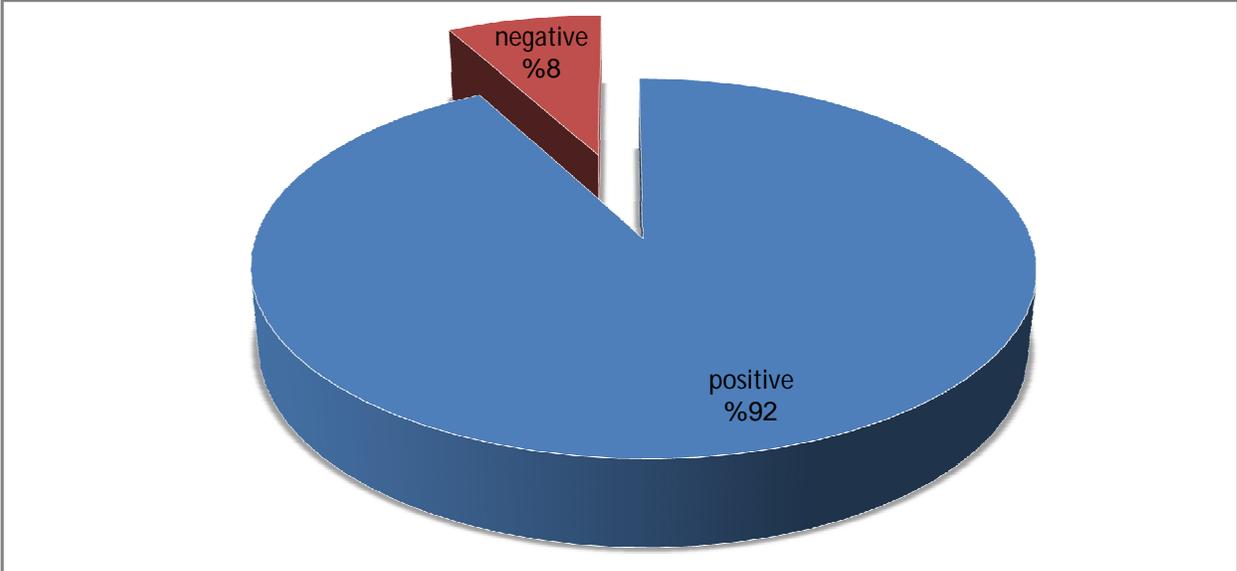


Figure 3-4: Frequency of Rh-D phenotype among the studied group.

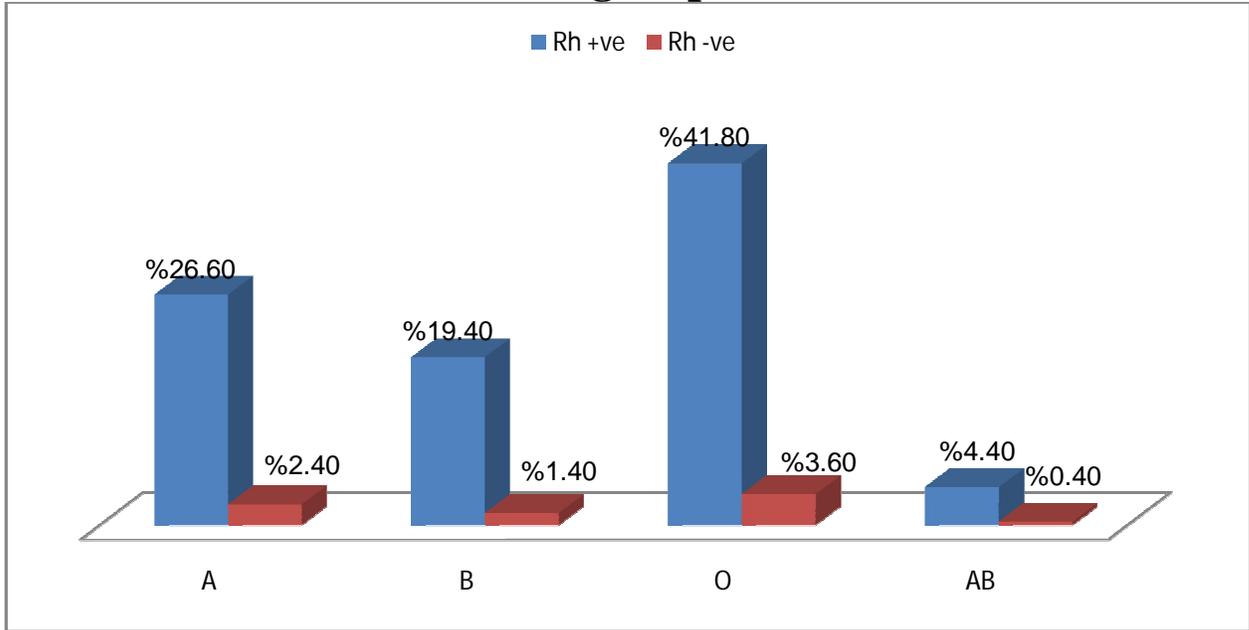


Figure 3-5: Frequency of ABO and Rh-D phenotype among the studied group.

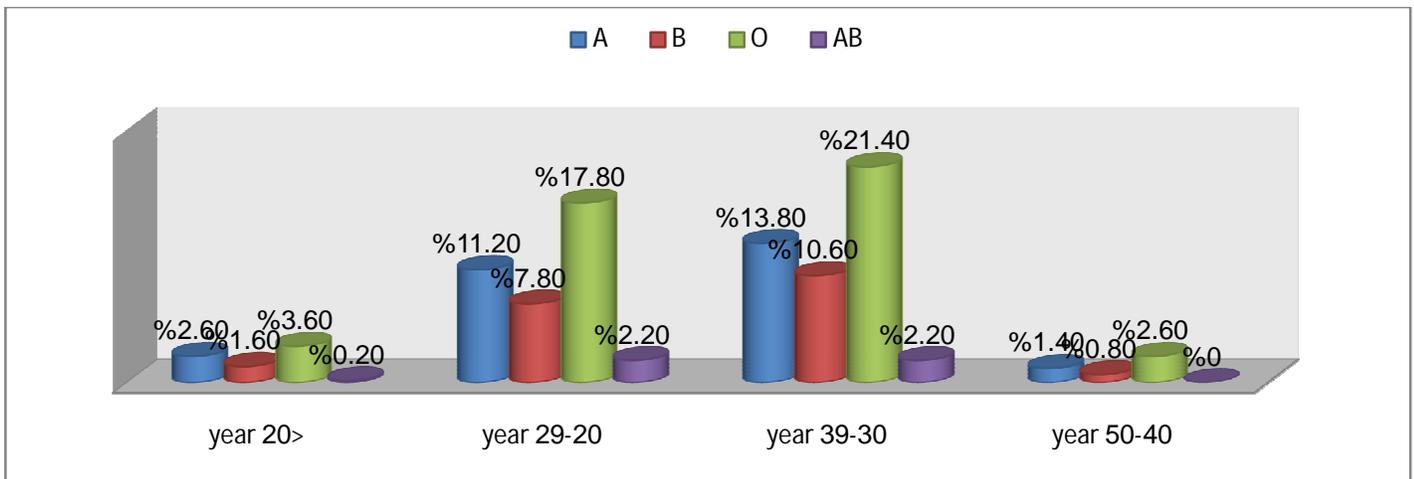


Figure 3-6: Frequency of ABO according to age group among studied group.

(p. value= 0.94)

Chapter four

4. Discussion, conclusion and recommendations

4.1. Discussion

This study was conducted in the Central Blood Bank in Khartoum State between October and November 2015, aimed to determine the frequency of 'ABO' and 'Rh-D' blood group antigens and phenotypes among Sudanese blood donors. Also it was attempted to compare between them and other populations.

The male donors were more than female donors with percent of 97% and 3% respectively. That may be due to the common culture that blood donation harm the health of women and the fear of anemia after blood donation. Most of the gender differences in donation patterns could be because of pregnancy and lactation.

In this study, the most frequent group of donors was age between 30 to 39 years (48%) followed by group aged 20 to 29 years (39%) then the group which aged less than 20 years (8%) and the least frequent group was aged between 40 to 50 years (4.8%).

Regarding to 'ABO' blood group system in this study, it was found that the most frequent antigen was 'O' with frequency of (45.4%) which was close to that found in Brazil (John, 2008) and Saudi Arabia (Bashwari *et al*,2001), while it was higher than that found in United states, United kingdom, Europe, India, and Germany. Overall, group 'O' is the most common blood type in the world (John, 2008).

The 'A' antigen frequency in this study was (29%) which was higher than that found in India and Saudi Arabia. Blood group 'A' is associated with high frequency in Europe especially in Scandinavia and central Europe, and its highest frequencies occur

in some Australian Aborigine population and black foot Indians of Montana (John, 2008).

The 'B' antigen was (20.8%) in this study which is higher than that found in most of European and Asian population. Blood group 'B' has its highest frequency in Northern India and neighboring Asian countries and its incidence diminishes both towards the west and the east, falling to single digit percentage in Spain. It is believed to have been entirely absent from Native American and Australian Aboriginal populations prior to the arrival of Europeans in these areas (John, 2008).

The lowest frequency of 'ABO' blood group was 'AB' antigen with percent of (4.8%) which is close to all population of the world in which the 'AB' blood group is also the lowest frequency (John, 2008).

The frequency of 'Rh-D' positive antigen was (92.2%) while 'Rh-D' negative antigen was (7.8%) in the current study which is close to all population of the world in which 'Rh-D' positive is also the higher frequency (John, 2008).

The frequency of 'A', 'B', 'O' and 'AB' groups in Rh-positive were (26.6%), (19.4%), (41.8%) and (4.4%), respectively. In Rh-negative the frequency of 'A', 'B', 'O' and 'AB' were (2.4%), (1.4%), (3.6%) and (0.4%), respectively.

The frequency of Rh-positive group in male subjects were (92.4%) where as in female subjects were (86.7%). About (7.6%) of male subjects and (13.3%) female subjects were found to be Rh-negative. These results are close to that found in Pakistan (Khattak, 2008).

The frequency of 'A', 'B', 'O', and 'AB' among male group were (29.3%), (20.8%), (45.2%) and (4.7%), respectively. In female group the frequency of 'A', 'B', 'O', and 'AB' were (20%), (20%), (53.3%) and (6.7%), respectively.

The frequency of 'A', 'B', 'O', and 'AB' among the group aged less than 20 years were as follow: (32.5%), (20%), (45%) and (2.5%), while in the group aged 20-29 years were (28.4%), (19.8%), (45.7%), and (6%). In the group aged 30-39 years the frequencies were (28.9%), (22.2%), (44.3%) and (4.6%). Finally, in the group aged 40-50 years the frequencies were (29%), (16.7%), (54.3%) and (0%), respectively.

4.2. Conclusion:

- Blood group 'O' was the most common phenotype in Sudanese donors, 'AB' blood group was the least phenotype in this study.
- The 'Rh-D' antigen was positive in (92.2%) and negative in (7.8%) among the studied group.
- Statistically there is no significant relationship between blood group and gender or age in this study.
- Considerable similarities exist between the group under study and different Arab, African and some South American populations.

4.3. Recommendations:

- It is necessary to determine blood groups of different ethnic groups and geographical areas to be useful in blood transfusion using large sample size.
- Determination of other blood group systems rather than that included in this study is encouraged.
- Correlation with results of viral infection screening (HIV, HBV, HCV) is recommended.

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Appendices

Appendix (1): Questionnaire

Sudan University of Science and Technology

Collage of graduate studies

Department of hematology and immunoematology

Frequency of ABO and Rhesus-D blood group antigens and phenotypes among Sudanese blood donors in Central Blood Bank, Khartoum State

Questionnaire No.

Date:

Gender: Male ()

Female ()

Age: ()

Blood group: A ()

B ()

O ()

AB ()

Rh-D: positive ()

negative ()