Chapter one
Introduction and literature review:

1.1 Introduction:

1.1.1 Background:

Blood-letting (venosection) was widely practised for a variety of medical conditions from the time of Hippocrates (≈ 430 BC) through to the nineteenth century in Europe and yet transfusion only became a commonplace therapeutic intervention less than 100 years ago. This is because both an understanding of the nature of blood as well the physiology of the circulation were required as a foundation for the development of blood transfusion and these were not forthcoming until the middle of the seventeenth century. The views of the Romans and ancient Greeks exerted a profound influence on both the traditions and practise of Western medicine for nearly 2000 years. The principal beliefs of the ancient Greeks and Romans were based on the writings of Hippocrates. The central doctrine of the humoral theory is set out in the treatise entitled ‘On the nature of man’, in which it was proposed that all living matter is composed of four basic ingredients, namely blood, phlegm, yellow bile and black bile (Lloyd, 1978). Initially involving transfusions from one animal to another and then transfusions from animals to man (Keynes, 1967). The first person credited with transfusing blood from one human to another was James Blundell, an obstetrician at Guy's and St. Thomas' Hospitals in London. He had seen many cases of postpartum hemorrhage and this stimulated research into blood transfusion using dogs. He showed that death from hemorrhage could be prevented in dogs by transfusion and venous blood was just as effective as arterial blood for resuscitation. He concluded that ‘only human blood should be employed’ after observing that dogs given human blood invariably died. He developed a syringe with a two-way stopcock and this was used with a

considerable degree of success to treat women with postpartum hemorrhage (Blundell, 1828; Jones & Mackmul, 1928).

Blood transfusion is generally the process of receiving blood products into one's circulation intravenously. Transfusions are used in a variety of medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood. Units of packed red blood cells are typically only recommended when a person's hemoglobin levels fall below 7g/dL. When a patient's own blood is salvaged and rein fused during a surgery (e.g. using a cell salvage machine such as a Cell Saver), this can be considered a form of auto-transfusion (and thus a form of transfusion) even though no "blood product" is actually created. (Carson *et al.*, 2010).

Blood and blood components are considered drugs because of their use in treating diseases. As with drugs, adverse effects may occur, necessitating careful consideration of therapy. The transfusion of blood cells is also transplantation, in that the cells must survive and function after transfusion to have a therapeutic effect. The transfusion of RBCs is the best-tolerated form of transplantation, may cause rejection as in a hemolytic transfusion reaction. The rejection of platelets as shown by refractoriness to platelet transfusion is relatively common in multiply transfused patients. Transfusion therapy is used primarily to treat two conditions: inadequate oxygen-carrying capacity because of anemia or blood loss, and insufficient coagulation proteins or platelets to provide adequate hemostasis (Landsteiner and Wiener., 1940).

1.1.2 Red Blood Cell Antigens:

Red blood cell antigens and corresponding antibodies provide the foundation for blood bank testing. Every individual’s red blood cells contain a unique genetically determined set of antigens. More than 20 blood group systems contain greater than 200 red blood cell antigens. The ABO and Rh antigens are

matched between donor and recipient. Additional red blood cell antigens are not considered in routine pre-transfusion testing unless a red cell stimulated antibody is present in the individual's plasma (Hoffbrand *et al.*, 2005).

1.1.3 Blood group antibodies:

Several terms have been used in the past and are still sometimes used to describe different types of blood group antibodies. These are 'naturally occurring' and 'immune' antibodies, 'cold' and 'warm' antibodies, and IgM, 'complete' (or saline) and IgG, 'incomplete' antibodies. Blood group antigen–antibody reactions: In blood group serology, the interaction between the antigen sites on the cells and the corresponding antibody is normally detected by observing agglutination of the cells concerned (Hoffbrand *et al*., 2005).

Philip Levine and Rufus Stetson recognized Alloimmunization to RBC antigens, a consequence of blood transfusion or pregnancy, following the discovery of the Rhesus (now Rh) blood group system in 1939. In 1945, Coombs, Mourant and Race described the use of antihuman globulin (later known as the "Coombs' test") to identify "incomplete" antibodies. A year later, they used this test to detect Rh antibodies on RBCs of babies suffering from HDN (Aygun *et al*., 2002).

Thereafter, the versatility of the Coombs' test in immunohematology for the detection of post-transfusion and maternal RBC alloantibodies became evident (Carson *et al*., 2010).

Antibody screening and identification: Patients' sera should be screened against unpooled group O cells from selected individuals known to carry the following antigens between them: D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b. Ideally, one cell sample should be R_1R_1 (DCe/DCe) and the other R_2R_2 (DcE/DcE), and a minimum homozygous expression of Fy^a and Jk^a should be present on one of the red cell samples. It is generally possible to meet these requirements with two cells but, if more antigens are required with

homozygous expression, it might be necessary to use three cells. The techniques employed for antibody screening need only include a well-controlled IAT, commonly now using micro column techniques. For antibody identification, a second sensitive technique, in addition to the IAT, such as the use of enzyme-treated cells, PEG or the manual polybrene tests, should be used. Saline tests are not essential and all incubations should be performed at 37°C; antibodies reacting at lower temperatures are of no clinical importance. It is recommended that an autologous control be used by incubating the patient's serum with the patient's cells, by the methods used for antibody identification. All tests must follow a written standard operating procedure. The antiglobulin or Coombs' test: The antiglobulin test is used to detect IgG antibodies that do not cause direct agglutination of red cells carrying the corresponding antigen when suspended in saline (Hardwick *et al.*, 2004).

1.2 Literature review:

Immunohematology as a defined term can be broken into two components: "immuno" is related to immune response and "hematology" is the study of blood (Harming .2005). The meaning of the term of immunity (from the Latin immunities –exemption from civic duties afforded to senators) as it is used today derived from earlier usage referring to exemption from military service or paying taxes (Weir and Stewart 1999).

Immunity: the body's defense against foreign or abnormal material, e.g. invading micro-organisms; immune responses are either specific, being mediated by cells that can recognize antigens, or nonspecific, e.g. mediated by complement in the absence of specific immune responses (Barbara and Rajeev 2003).

Immunization: the process by which immunity to various infections is promoted by exposure to altered or killed microorganisms or antigens derived from them (Barbara and Rajeev 2003).

Alloimmunization: is a reaction of the immune system to foreign antigens. It is one of the most important complications of blood transfusions. In the presence of alloantibody, the life span of red blood cells is shortened and the patient's need for blood increases. Identification of the types of antigens present and transfusion of fully compatible blood may prevent alloimmunization (Aygun *et al* ,2002).

Antigens: was defined as the part of a molecule that is bound by a specific antibody.More recently, it has become customary to define an antigen as a substance that can stimulate an immune response (immunogenicity) (Hoffbrand *et al*., 2005).

Immune responses can be either positive or negative. Positive responses lead to the production of antibodies (humoral immunity) and/or proliferation of immunocompetent cells (cellular immunity) that can bind and eliminate their

stimulatory antigen. In negative responses, the cells that mediate humoral and cellular immune responses are rendered non-responsive (Hoffbrand *et al*., 2005).

Antibodies: are immunoglobulins (Ig) produced by the B lymphocytes of the adaptive immune system in response to an antigen for which they exhibit specific binding (Hoffbrand *et al*., 2005).

Depending on the origin of the antigenic stimulus, antibodies can be termed: (i) alloantibodies, when produced by an individual against epitopes present in another individual of the same species; (ii) autoantibodies, when reactive with determinants present on the individual's own antigens; and (iii) heteroantibodies (or xenoantibodies), when produced against antigenic determinants present on the cells of another species (Hoffbrand *et al*., 2005).

1.2.1 The discovery of blood groups :

Karl Landsteiner truly opened the doors of blood banking with his discovery of the first human blood group system. This marked the beginning of the concept of individual uniqueness defined by the RBC antigens present on the RBC membrane. The ABO system is the most important of all blood groups in transfusion practice (Harming 2005).

1.2.2 ABO Blood Groups:

Bernstein first described the theory for the inheritance of the ABO blood groups in 1924; he demonstrated that an individual inherits one ABO gene from each parent and that these two genes determine which ABO antigens are present on the RBC membrane (Harming 2005) .

The inheritance of ABO genes, therefore, follows simple Mendelian genetics. ABO, like most other blood group systems, is co dominant in expression one position, or locus, on each chromosome 9 is occupied by an *A*, *B*, or *O* gene (Harming 2005).

1.2.2.1 A, B, and H RBC Antigens:

The formation of ABH antigens results from the interaction of genes at three separate loci (ABO, Hh, and Se). These genes do not actually code for the production of antigens but rather produce specific glycosyltransferases that add sugars to a basic precursor substance. A, B, and H antigens are formed from the same basic precursor material (called a paragloboside) to which sugars are attached in response to specific enzyme tranferases elicited by an inherited gene. The precursor substance on erythrocytes is referred to as type 2; this means that the terminal galactose on the precursor substance is attached to the *N*-acetyl glucosamine in a beta 1 → 4 linkage (Harming 2005). A type 1 precursor substance refers to beta 1 → 3 linkages between galactose and *N* acetyl glucosamine. ABH antigens on the RBC are constructed on oligosaccharide chains of type 2 precursor substances, the ABH antigens develop as early as the 37th day of fetal life but do not increase much in strength during the gestational period. The RBCs of the newborn have been estimated to carry anywhere from 25 to 50 percent of the number of antigenic sites found on the adult RBC. As a result, reactions of newborn RBCs with ABO reagent antisera are frequently weaker than reactions with adult cells. The expression of A and B antigens on the RBCs is fully developed by 2 to 4 years of age and remains constant for life. As well as age, the phenotypic expression of ABH antigens may vary with race, genetic interaction, and disease states (Harming 2005).

1.2.2.2 Interaction of Hh and ABO Genes:

Individuals who are blood group O inherit at least one H gene (genotype HH or Hh) and two O genes. The H gene elicits the production of an enzyme, α-2-L-fucosyltransferase, which transfers the sugar L-fucose to an oligosaccharide chain on the terminal galactose of type 2 chains. Therefore, L-fucose is the sugar responsible for H specificity (blood group O) .The O gene at the ABO

locus, which is sometimes referred to as a morph, does not elicit the production of a catalytically active polypeptide, and therefore the H substance remains unmodified as a result, the O blood group has the highest concentration of H antigen. The H substance (L-fucose) must be formed for the other sugars to be attached in response to an inherited A and/ or B gene, the H gene is present in more than 99.99 percent of the random population. The allele of H, "h," is quite rare, and the genotype, hh, is extremely rare. The term "Bombay" has been used to refer to the phenotype that lacks normal expression of the ABH antigens because of the inheritance of the hh genotype, the hh genotype does not elicit the production of α-2-Lfucosyltransferase; as a result, L-fucose is not added to the type 2 chain, and H substance is not expressed on the RBC, even though Bombay (hh) individuals may inherit ABO genes. In the formation of blood group A, the A gene (AA or AO) codes for the production of α-3-N-acetylgalactosaminyltransferase, which transfers an N-acetyl-D galactosamine (GalNAc) sugar to the H substance. This sugar is responsible for A specificity (blood group A), the A-specific immunodominant sugar is linked to a type 2 precursor substance that now contains H substance through the action of the H gene. Individuals who are blood group B inherit a B gene (BB or BO) that codes for the production of α-3-D-galactosyltransferase, which then attaches D-galactose (Gal) sugar to the H substance previously placed on the type 2 precursor substance through the action of the H gene, this sugar is responsible for B specificity (blood group B). When both A and B genes are inherited, the B enzyme (α-3-Dgalactosyltransferase) seems to compete more efficiently for the H substance than the A enzyme (α-3-N-acetylgalactosaminyltransferase) (Harming 2005).

1.2.2.3 A, B, and H Soluble Antigens:

ABH antigens are integral parts of the membranes of RBCs, endothelial cells, platelets, lymphocytes, and epithelial cells. ABH-soluble antigens can also be

found in all body secretions, their presence is dependent on the ABO genes inherited as well as the inheritance of another set of genes (secretor genes) that regulate their formation. The inheritance of an Se gene codes for the production of a transferase (α-2-L-fucosyltransferase) that results in the modification of the type 1 precursor substance in secretions to express H substance. This H substance can then be modified to express A and B substance (if the corresponding gene is present) in secretions such as saliva (Harming 2005).

1.2.2.4 A Subgroups:

In 1911 von Dungern described two different A antigens based on reactions between group A RBCs and anti-A and anti-A_1, group A RBCs that react with both anti-A and anti-A_1 are classified as A_1 whereas those that react with anti-A and not anti-A_1 are classified as A_2. RBCs from A_1 and A_2 individuals react equally strong with reagent anti-A in ABO forward typing tests. Inheritance of an A_1 gene elicits production of high concentrations of the enzyme α-3-*N*acetylgalactosaminyltransferase, which converts almost all of the H precursor structure to A_1 antigens on the RBCs. A_1 is a very potent gene that creates from 810,000 to 1,170,000 antigen sites on the adult RBC, whereas inheritance of an A_2 gene results in the production of only 240,000 to 290,000 antigen sites on the adult A_2 RBC. The immunodominant sugar on both A_1 and A_2 RBCs is *N*-acetyl-D-galactosamine, qualitative differences also exist, inasmuch as 1 to 8 percent of A_2 individuals produce anti-A_1 in their serum, and 22 to 35 percent of A2B individuals produce anti-A_1 .This antibody can cause discrepancies in ABO testing and incompatibilities in cross matches with A_1 or A_1B cells, because anti-A_1 is a naturally occurring IgM cold antibody, it is unlikely to cause a transfusion reaction because it usually reacts better or only at temperatures well below 37 C^0, It is to be considered clinically significant if it is reactive at 37C^0 (Harming 2005).

1.2.2.5 Antibodies of the ABO system:

Sera taken from people over the age of about 6 months which do not contain the expected A and B antibodies are very rare. They should always be investigated thoroughly; often, some interesting explanation will be found, for example a rare subgroup of A, a blood group chimera or congenital absence of IgM. It is likely that ABO antibodies arise in response to A- and B like antigens present on bacterial, viral or animal molecules. Titers of ABO antibodies vary considerably with age, reaching a peak in young adults and then declining in old age. Naturally occurring anti-A and -B are antibodies that react better at lower temperatures than at 37°C. The antibodies always have some IgM component and, in group A and B persons, they are almost entirely IgM. However, the antibodies from group O individuals, even before immunization, usually have some IgG anti-A, B, an antibody that cross-reacts with both A and B structures. Persons of the appropriate ABO group can produce immune anti-A and -B after immunization with red cells or blood group substances. Immune anti-A and anti-B can also arise after various vaccinations and inoculations for the prophylaxis of infections. Here, the A-like antigens come from the hog pepsin digest used in their preparation. Following immunization, the thermal characteristics of the antibodies change, but group A and B subjects continue to produce antibodies that are mainly IgM. Most group O persons, however, will produce IgG as readily as IgM anti-A, B. This correlates with the fact that mothers of children with ABO hemolytic disease of the newborn (HDN) are usually group O. Immune anti-A, B are mainly IgG2, which does not cause HDN because there are no Fc receptors for IgG2 on the cells of the mononuclear phagocyte system. When the maternal serum contains potent IgG1 and/or IgG3 ABO antibodies, HDN may occur, although this is usually mild compared with Rh HDN. Some IgA anti-A or -B is produced following immunization with A or B substances.

Some differences in the serological properties of immune and naturally occurring anti-A and –B are shown, which also gives an indication to ways of detecting IgG anti-A or -B in the presence of IgM anti-A or -B, which has relevance to ABO HDN (Hoffbrand *et al*., 2005).

1.2.3 The Rh blood group:

The fourth system to be discovered, is the second most important in blood transfusion (Hoffbrand *et al.*, 2005).

1.2.3.1 The D antigen (Rh1):

In 1939, Levine and Stetson reported that a patient, who had delivered a stillborn infant and then suffered a severe reaction to transfusion of her husband's blood, had an antibody that agglutinated the red cells of 85% of ABO-compatible donors. In 1940, Landsteiner and Wiener found that guinea pigs and rabbits injected with rhesus monkey red cells made an antibody that not only agglutinated rhesus monkey red cells, but also the red cells of 85% of people of European origin. Many years later, it was realized that the human antibody (now called anti-D of the Rh system) does not identify the same antigen as the rabbit and guinea pig rhesus antibody, the error arising out of a phenotypic association between the antigens. As it was now too late to change the name of the whole system, Levine suggested that the antigen defined by the original rhesus antibody should be called LW in honour of Landsteiner and Wiener (Hoffbrand *et al*., 2005).

1.2.3.2 C, E, c and e (Rh2 to Rh5):

By the end of 1943, four antisera detecting genetically related antigens were available to Fisher and Race, who noticed that two of them appeared to give antithetical results. They proposed that the antigens recognized by these two antisera were allelic and called them C and c. They gave further letters, D (the original Rh antigen) and E, to the antigens recognized by the other two antisera and postulated that each had an alternative, which they called d and e. Anti-e

was found in 1945, anti-d has never been found as no d antigen exists. Fisher and Race proposed that three closely linked genes, giving rise to eight gene complexes or haplotypes, controlled the Rh antigens: CDe, cDE, cDe, CDE, cde, Cde, cdE and CdE. At about the same time, Wiener proposed that there was only one Rh gene, controlling a number of blood factors, equivalent to C, c, D, E and e . Molecular genetics has shown that there are two Rh genes, one encoding D, the other encoding the Cc and Ee antigens. However, as the Cc and Ee polymorphisms are determined by separate regions of a single gene, the CDE terminology of Fisher and Race is still suitable for understanding Rh at most levels (although the Wiener terminology is often used as a shorthand). Genotype frequencies vary considerably in different parts of the world (Hoffbrand *et al*., 2005).

1.2.3.3Antibodies of the Rh system:

Generally, Rh antibodies are only produced following immunization by red cells. However, anti-E is often naturally occurring; about one-half may occur without a history of pregnancy or transfusion. Rarely, naturally occurring anti-D and anti-C^w are found and such antibodies react optimally with enzyme-treated cells (Hoffbrand *et al*., 2005).

The clinical importance of the Rh system lies in the readiness with which anti-D arises after stimulation with D-positive red cells by pregnancy or transfusion. Prophylaxis of Rh immunization with anti-D immunoglobulin has led to a significant decrease in the incidence of anti-D, but it remains the most common immune antibody of clinical relevance detected in a routine blood transfusion laboratory. D is considerably more immunogenic than the other Rh antigens, which have the following order of immunogenicity: c > E > e > C. About 20–30% of anti-D sera also appear to contain anti-C. Usually, this anti-C is not a separable antibody and is probably more correctly called anti-G. About 1–2% of anti-D sera also contain anti-E. Anti-C (and anti-G) in the absence of anti-D

is very uncommon. The incidence of other Rh antibodies is much lower, but together they are more common than the antibodies against K (Kell), which is the most immunogenic antigen after D. In routine screening, pure anti-E is the most common, followed by anti-c, although anti-c is a more common cause of HDN, which can be severe. This is probably because about one-half of the examples of anti-E are weak, naturally occurring antibodies. Anti-e, like anti-C, is very rare. The vast majority of Rh antibodies are IgG and do not fix complement. Anti-D may occasionally be partly IgA. IgM anti-D is very rare (Hoffbrand *et al*., 2005).

1.2.4 Other blood groups:

1.2.4.1 The Lewis system:

The Lewis system differs from all other blood group systems in that it is primarily a system of soluble antigens present in secretions and in plasma. The Lewis antigens on red cells are adsorbed passively from the plasma, and the constant presence of plasma is needed to maintain Lewis antigen on the red cells. There are two basic Lewis antigens; Le^a and Le^b. Expression of either requires the presence of an active Lewis gene, but the gene controlling H secretion (FUT2) also governs Lewis phenotypes. Lewis antigens in saliva and plasma are glycoproteins and glycolipids respectively (Hoffbrand. et al, 2004).

The Lewis gene, *FUT3*, encodes an α1,4-fucosyltransferase that catalyses the addition of l-fucose in 1→4 linkage to the subterminal *N*-acetylglucosamine of type 1 chains, If the type 1 core structure has been unmodified, Le^a antigen is produced (Hoffbrand *et al.*, 2005).

Adsorption of the Lewis substances by red cells: Le (a+b-) and Le (a-b+ red cells incubated in Le (a-b-) plasma lose their Lewis antigens into the plasma. Similarly, if Le (a+b-) or Le (a-b+) red cells are transfused to Le (a-b-) person, the transfused cells will gradually lose the Le^a or Le^b antigens and will group as Le (a-b-) within 1 week of transfusion. So, if the patient has anti-Le^a or -Le^b,

the red cells that were not destroyed in the first few days, will not be haemolysed once they become Le (a-b-) (Hoffbrand *et al.*, 2005).

Clinical significance of Lewis antibodies: Some patients may have Lewis antibodies reacting at 37°C. Anti- Le^a is usually more hemolytic than anti-Le^b, and there are some anti-Le^{a+b} that can be very potent, leading to increased intravascular red cell destruction in the initial stages of transfusion. However, all Lewis antibodies lead to two-component survival curves of transfused incompatible red cells; the first cells are destroyed at an accelerated rate and the remainder will have a normal survival. The red cells that survive normally have been stripped of their adsorbed Lewis antigens and have become Le (a-b-), the same as the recipient. It is therefore recommended that, for patients with Lewis antibodies reacting at 37°C, ABO identical red cells compatible in an IAT cross-match at 37°C should be transfused. The provision of pre-typed Le (a-b-) blood for patients with Lewis antibodies is not necessary, as it is always easy to find cross match-compatible red cells at 37°C. Lewis antibodies do not cause HDN as they are usually IgM and newborn infants have Le (a-b-) red cells (Hoffbrand *et al.*, 2005).

1.2.4.2 The MNS system:

Landsteiner and his colleagues discovered MNS in 1927 and the MNS genes were the first blood group genes to be cloned, in 1986 and 1987. The MNS system now contains 43 antigens, but is relatively unimportant in clinical blood transfusion. M and N are inherited as codominant Mendelian traits, The *Ss* locus, which is closely linked to *MN*, also consists of two codominant alleles. The MN antigens are carried on glycophorin A (GPA), which is encoded by the *GYPA* gene on chromosome 4. M and N differ by amino acids at positions 1 and 5 of the external N-terminus of GPA. Glycophorin B (carries the S and s determinants, which represent an amino acid substitution at position 29. GPB is encoded by *GYPB*, which is closely linked and homologous to *GYPA*. S-s-U--

red cells lack GPB. The amino acid sequence at the N-terminus of GPB is identical to that of N-specific GPA and accounts for the weak reactivity of *M/M* cells with anti-N, provided they are S+or s. GPA and GPB are exploited as receptors by the malaria parasite *Plasmodium falciparum* (Hoffbrand *et al*., 2005).

Anti-M is uncommon and reacts with about 80% of random samples. It is naturally occurring, more common in infants than adults, but can be immune and can very rarely cause HDN. Anti-N is also rare and reacts with about 70% of random samples. It is nearly always a cold-reactive IgM antibody. Because of the 'N' activity of GPB, at low temperatures anti-N reacts with, and can be completely absorbed by, *M/M* cells, except those of the M+N-S-s-phenotype. Useful anti-N lectin can be prepared from the seeds of *Vicia graminea*. A cold-reacting N-like antibody (anti-Nf) has been described in certain patients undergoing renal dialysis, regardless of their MN group. Although it may cause confusion in cross-matching tests, anti-Nf is usually of little clinical importance, but has been reported to cause hyper acute renal graft rejection. NF arises from the effects of minute amounts of formaldehyde (used to sterilize the dialyzer coil) on the patient's red cells; these changed cells stimulate anti-Nf. Anti-S, the rarer anti-s and anti-U are usually immune, IgG, and can cause HDN. They have also been implicated in HTRs. Anti-U only occurs in S-s-black people and reacts with all cells that have the S or s antigens and up to 50% of cells that are S-s-. Finding compatible blood for a patient with anti-U may prove difficult (Hoffbrand *et al.*, 2005).

1.2.4.3 The Lutheran blood group system:

Lutheran is a complex system comprising four pairs of allelic antigens: Lu^a and Lu^b, Au^a and Au^b, Lu^6 and Lu^9, Lu^8 and Lu^{14}. The incidence of Lu^a and Lu^b phenotypes in the UK population is as follows: Lu (a+b-) 0.1%; Lu (a-b-) 7.5%; Lu (a-b+) 92.4%. The rare Lu^{null} phenotype, in which no Lutheran

antigens are expressed. Lutheran antibodies are uncommon and are not generally considered clinically significant, although anti-Lu^b may have caused mild delayed HTRs. Lu^a may be omitted from antibody screening cells. The Lutheran glycoproteins bind the extracellular matrix glycoprotein laminin and might function as adhesion molecules (Hoffbrand *et al.*, 2005).

1.2.4.4 The Kell blood group system:

The Kell system consists of one triplet and four pairs of allelic antigens – K and k; Kp^a, Kp^b, and Kp^c; Js^a and Js^b; K^{11} and K^{17}; K^{14} and K^2 – all of which represent amino acid substitutions in the Kell glycoprotein, plus 11 high frequency and three low frequency antigens. The incidence of the K/k phenotypes, the most important clinically, is as follows: K+k- 0.2%; K+k+ 8.7%; K-k+ 91.1%. K is rare in populations other than those of white people. Js^a is present in about 20% of black people, but is extremely rare in other ethnic groups. The very rare null phenotype of the Kell system, in which no Kell system antigens are expressed, is called K_o. Anti-K is an important antibody in white populations; it is nearly always immune, IgG and complement-binding. It has been the cause of severe HTRs and HDN. K antigen stimulates the formation of anti-K in about 10% of K-negative people who are given one unit of K-positive blood. This makes K the next most immunogenic antigen after D. About 0.1% of all cases of HDN are caused by anti-K; most of the mothers will have had previous blood transfusions. HDN caused by anti-K differs from Rh HDN in that anti-K appears to cause fetal anemia by suppression or erythropoiesis, rather than immune destruction of mature fetal erythrocytes. Kell antigen is expressed by erythroid cells at a very early stage of erythropoiesis and anti-K probably facilitates immune destruction of early erythroid progenitors, before they become haemoglobinized. Anti-K is best detected by the IAT; anti-K does not always agglutinate red cells treated with enzymes, or suspended in low-ionic-strength solution. Anti-k is a very rare

antibody, which reacts with 99.8% of random blood samples. It is always immune and has been incriminated in some cases of mild HDN. Most other Kell system antibodies are rare and best detected by the IAT (Hoffbrand *et al*., 2005).

1.2.4.5 The Duffy blood group system:

The allelic antigens Fy^a and Fy^b represent a single amino acid substitution in the extracellular N-terminal domain of the Duffy glycoprotein. Their incidence in the UK is as follows: Fy (a+/-) 20%; Fy (a-b-) 46%; Fy (a-b+) 34%. About 70% of African-Americans and close to 100% of West Africans are Fy (a-b-). They are homozygous for Fy^b allele containing a mutation in a binding site for the erythroid-specific GATA-1 transcription factor, which means that Duffy glycoprotein is not expressed in red cells, although it is present in other tissues. The Duffy glycoprotein is the receptor essential for penetration of *P. vivax* merozoites into erythroid cells and the Fy (a-b-) phenotype confers resistance to *P. vivax* malaria. The Duffy glycoprotein (also called Duffy-Antigen Chemokine Receptor, DARC) is a red cell receptor for a variety of chemokines, including interleukin 8. It might function as a 'sink' or scavenger for the removal of unwanted chemokines. Anti-Fy^a is not infrequent and is found in previously transfused patients who have usually already made other antibodies. It is IgG, often complement fixing and can cause HTRs, but seldom HDN. It is best detected by IAT and does not react with red cells treated with the proteases papain and ficin. Anti-Fy^b is very rare and is always immune (Hoffbrand *et al*., 2005).

1.2.4.6 The Kidd blood group system:

Kidd has two alleles, Jk^a and Jk^b, which represent a single amino acid change in the Kidd glycoprotein. Phenotype frequencies in the UK population are as follows: Jk (a+b-) 25%; Jk (a-b-) 50%; Jk (a-b+) 25%. A Kidd-null phenotype, Jk (a-b-), results from homozygosity for inactivating mutations in the Kidd

gene, *SLC14A1*. It is very rare in most populations. The Kidd glycoprotein is a urea transporter in red cells and in renal endothelial cells. Anti-Jk^a is uncommon and anti-Jk^b is very rare, but they may both cause severe transfusion reactions and, to a lesser extent, HDN. Kidd antibodies have often been implicated in delayed HTRs; they are IgG and predominantly complement fixing, but may be difficult to detect because they tend to disappear and then reappear promptly in anamnestic responses. Patients who have made Kidd antibodies should always be given an antibody card (Hoffbrand *et al*., 2005).

1.2.4.7 P blood groups:

Landsteiner and Levine, who used suitably absorbed sera of rabbits injected with human red cells, discovered P1. About 75% of subjects tested were positive for P1, which is inherited as a Mendelian dominant character. P1 frequency varies in different populations and the P1-negative phenotype is called P2. P1 is weakly expressed at birth and its strength varies considerably in adults. For this reason, identification of anti-P1 can be difficult, as panel cells will have varying expression of the antigen. Anti-P1 is a naturally occurring antibody commonly found in the serum of P2 individuals. Unlike anti-A and -B, anti-P1 rarely causes transfusion reactions because it is usually a cold-reacting IgM antibody, often not reactive above 30°C. Potent anti-P1 can be found in patients with hydatid disease and can be inhibited by hydatid cyst fluid. PK red cells may be P1 or P2. Anti-PP1PK and anti-P invariably occur in the sera of the very rare individuals with p and PK phenotypes respectively. Anti- PP1PK reacts with all red cells except those of the p phenotype; anti-P reacts with all red cells except those of the PK and p phenotypes. They are usually strong IgM antibodies, are often lytic at 37°C, and can cause severe transfusion reactions if incompatible red cells are transfused. Occasionally, IgG anti-P or anti-PPl PK are found and have been associated with spontaneous early abortion. The biphasic Donath–Landsteiner antibody is found in the sera

of patients suffering from paroxysmal cold haemoglobinuria. It is always IgG, usually has anti-P specificity and is an autoantibody (Hoffbrand *et al*., 2005).

1.2.5 Blood transfusion:

Blood transfusion is generally the process of receiving blood products into one's circulation intravenously. Transfusions are used in a variety of medical conditions to replace lost components of the blood (Carson *et al*., 2010).

1.2.5.1 Sources of blood transfusion:

Blood transfusions typically use sources of blood:

1.2.5.1 .1 Homologous transfusion (allogeneic transfusion):

Homologous transfusion is the allogenic blood transfusion from one person (donor) to another (receptor), and the precondition is compatibility of blood types between the donor and receptor (Ritter *et al*., 1994).

1.2.5.1 .2 Autologous transfusion:

Autologous transfusion is a process wherein a person receives their own blood for a transfusion, instead of banked allogenic (separate-donor) blood. There are two main kinds of Autologous transfusion: Blood can be autologously "pre-donated" (termed so despite "donation" not typically referring to giving to one's self) before a surgery, or alternatively, it can be collected during and after the surgery using an intraoperative blood salvage device (such as a Cell Saver). The latter form of Autologous transfusion is utilized in surgeries where there is expected a large volume blood loss - e.g. aneurysm, total joint replacement, and spinal surgeries (Carey 1987).

The first documented use of autologous blood transfusion was in 1818 when an Englishman, Blundell, salvaged vaginal blood from patients with postpartum hemorrhage. By swabbing the blood from the bleeding site and rinsing the swabs with saline, he found that he could re-infuse the result of the washings. This unsophisticated method resulted in a 75% mortality rate, but it marked the start of autologous blood transfusion (Blundell, 1918).

Medical indication: Autotransfusion is intended for use in situations characterized by the loss of one or more units of blood and may be particularly advantageous for use in cases involving rare blood groups, risk of infectious disease transmission, restricted homologous blood supply or other medical situations for which the use of homologous blood is contraindicated. Autotransfusion is commonly used intraoperatively and postoperatively. Intraoperative autotransfusion refers to recovery of blood lost during surgery or the concentration of fluid in an extracorporeal circuit. Postoperative autotransfusion refers to the recovery of blood in the extracorporeal circuit at the end of surgery or from aspirated drainage (Shiley, 1988) .

Contraindications: The use of blood recovered from the operative field is contraindicated in the presence of bacterial contamination or malignancy. The use of autotransfusion in the presence of such contamination may result in the dissemination of pathologic microorganisms or malignant cells (Shiley, 1988).

1.2.6 Blood components:

A blood donation is taken by an aseptic technique into plastic bags containing an appropriate amount of anticoagulant-usually citrate, phosphate, dextrose (CPD). The citrate anticoagulants the blood by combining with the blood calcium. Three components are made by initial centrifugation of whole blood: red cells, buffy coat and plasma (Hoffbrand *et al*., 2006).

1.2.6.1 Red cells:

Packed (plasma-depleted) red cells are the treatment of choice for most transfusions .In older subjects, a diuretic is often given simultaneously older subjects, a diuretic is often given simultaneously and the infusion should be sufficiently slow to avoid circulatory overload. Iron chelation therapy should be considered with patients on a regular transfusion programme to avoid iron overload Recombinant erythropoietin is widely used to reduce transfusion requirements (e.g. in patients on dialysis, cancer patients and myelodysplasia).

Factor VIla can reduce transfusion need in patients with major haemorrhage (e.g. at surgery or after trauma). Red cells are stored at 4-6°C for up to to 35 days, depending on the preservative. After the first 48 h there is a slow progressive K+ loss from the red cells into the plasma. In cases where infusion of K+ could be dangerous, fresh blood should be used (e.g. for exchange transfusion in haemolytic disease of the newborn). During red cell storage there is a fall in 2,3- diphosphoglycerate (2,3-DPG) but after transfusion 2,3DPG levels return to normal within 24 h . Optimum additive solutions have been developed to increase the shelf life of plasma-depleted red cells by maintaining both adenosine triphosphate (ATP) and 2,3-DPG levels (Hoffbrand *et al*., 2006).

1.2.6.2 Leucodepletion :

blood products are now routinely filtered to remove the majority of white cells, a process known as leucodepletion. This is usually performed soon after collection and prior to processing and is more effective than filtration of blood at the bedside. A blood component is defined as leucocyte depleted if there are less than 5 x 106/L white cells present.

Leucodepletion reduces the incidence of febrile transfusion reactions and HLA alloimmunization. It is effective at preventing transmission of CMV infection (Hoffbrand *et al*., 2006). .

1.2.6.3 Granulocyte concentrates:

These are prepared as buffy coats or on blood cell separators from normal healthy donors or from patients with chronic myeloid leukemia. They have been used in patients with severe neutropenia ($<0.5x10^9$/L) who are not responding to antibiotic therapy but it is not usually possible to give sufficient amounts. They may transmit CMV infection and must be irradiated to eliminate the risk of causing GVHD (Hoffbrand *et al*., 2006).

1.2.6.4 Platelet Concentrates:

These are harvested by cell separators or from individual donor units of blood. They are stored at room temperature. Platelet transfusion is used in patients who are thrombocytopenic or have disordered platelet function and who are actively bleeding (therapeutic use) or are at serious risk of bleeding (prophylactic use). For prophylaxis, the platelet count should be kept above 5-10 x 10^9/L unless there are additional risk factors such as sepsis, drug use or coagulation disorders for which the threshold should be higher. Therapeutic use is indicated in bleeding associated with platelet disorders. In massive haemorrhage the Count should be kept above 50 x 10^9/L. Platelet transfusions should be avoided in autoimmune thrombocytopenic purpura unless there is serious haemorrhage. They are contraindicated in heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. Refractoriness to platelet transfusions is defined by a poor platelet increment post-transfusion (7.5 x 10^9/L at 1 h or <4.5 x 10^9/L at 24 h). The causes are either immunological (mostly HLA alloimmunization) or non-immunological (sepsis, hypersplenism, DIC, drugs). Platelets express HLA class I (but not class II) antigens and HLA-matched or cross-match-compatible platelets are needed for patients with HLA antibodies (Hoffbrand *et al*., 2006).

1.2.6.5 Preparations from human plasma:

1.2.6.5.1 Fresh frozen plasma (FFP):

Rapidly frozen plasma separated from fresh blood is stored at less than -30°C. Frozen plasma is usually prepared from single donor units though pooled products are also available. Its main use is for the replacement of coagulation factors (e.g. when specific concentrates are unavailable) or after massive transfusions, in liver disease and DIC, after cardiopulmonary bypass surgery, to reverse a warfarin effect, and in thrombotic thrombocytopenic purpura. Virally inactivated forms of FFP are now available (Hoffbrand *et al*., 2006).

1.2.6.5.2 Human albumin solution (4.5%):

It is a useful plasma volume expander when a sustained osmotic effect is required prior to the administration of blood, but it should not be given in excess, It is also used for fluid replacement in patients undergoing plasmapheresis and sometimes for fluid replacement in selected patients with hypoalbuminaemia (Hoffbrand *et al*., 2006).

1.2.6.5.3 Human albumin solution (20%) (salt-poor albumin):

It may be used in severe hypoalbuminaemia when it is necessary to use a product with minimal electrolyte content. Principal indications for its use are patients with nephrotic syndrome or liver failure (Hoffbrand *et al.*, 2006).

1.2.6.5.4 Cryoprecipitate:

This is obtained by thawing FFP at 4°C and contains concentrated factor VIII and fibrinogen, It is stored at less than -30°C or, iflyophylized, at 4-6°C, and was used widely as replacement therapy in haemophilia A and von Willebrand disease before more purified preparations of factor VIII became available, Its main use is m fibrinogen replacement in disseminated intravascular coagulation (DIC) or massive transfusion or hepatic failure (Hoffbrand *et al*., 2006).

1.2.6.5.5 Freeze-dried factor VIII concentrates:

These are also used for treating haemophilia A or von Willebrand disease. The small volume makes them ideal for children, surgical cases, patients at risk from circulatory overload and for those on home treatment. Their use is declining as recombinant forms of factor VIII become widely available (Hoffbrand *et al.*, 2006).

1.2.6.5.6 Freeze-dried factor IX-prothrombin complex concentrates :

A number of preparations are available that contain variable amounts of factors II, VII; IX and X. They are mainly used for treating factor IX deficiency (Christmas disease) but are also used in patients with liver disease or in

haemorrhage following overdose with oral anti-coagulants or in patients with factor VIII inhibitors. There is a risk of thrombosis (Hoffbrand *et al.*, 2006).

1.2.6.5.7 Protein C concentrate:

This is used in severe sepsis with disseminated intravascular coagulation (e.g. meningococcal septicaemia) to reduce thrombosis resulting from depletion of protein C (Hoffbrand *et al.*, 2006).

1.2.6.5.8 Immunoglobulin:

Antibodies against common viruses. It is used in Pooled immunoglobulin is a valuable source of hypogammaglobulinaemia for protection against viral and bacterial disease. It may also be used in immune thrombocytopenia and other acquired immune disorders (e.g. post-transfusion purpura or alloimmune neonatal thrombocytopenia) (Hoffbrand *et al.*, 2006).

1.2.6.5.9 Specific immunoglobulin:

This may be obtained from donors with high titres of antibody (e.g. anti-RhD, antihepatitis B, antiherpes zoster or antirubella) (Hoffbrand *et al.*, 2006).

1.2.7 Compatibility testing:

Before a recipient receives a transfusion, compatibility testing between donor and recipient blood must be done. The first step before a transfusion is given is to Type and Screen the recipient's blood. Typing of recipient's blood determines the ABO and Rh status. The sample is then screened for any alloantibodies that may react with donor blood (Carson *et al.*, 2010).

A positive screen warrants an antibody panel/investigation to determine if it is clinically significant. An antibody panel consists of commercially prepared group O red cell suspensions from donors that have been phenotyped for commonly encountered and clinically significant alloantibodies (Carson *et al.*, 2010).

If there is no antibody present, an immediate spin cross match or computer assisted cross match is performed where the recipient serum and donor serum

are incubated. In urgent cases where cross matching cannot be completed, and the risk of dropping hemoglobin outweighs the risk transfusing uncross matched blood, O-negative blood is used, followed by cross match as soon as possible. O-negative is also used for children and women of childbearing age. It is preferable for the laboratory to obtain a pre-transfusion sample in these cases so a type and screen can be performed to determine the actual blood group of the patient and to check for alloantibodies (Carson *et al*., 2010).

1.2.7.1 Complications of blood transfusion:

Transfusions of blood products are associated with several complications, many of which can be grouped as immunological or infectious. There is also increasing focus (and controversy) on complications arising directly or indirectly from potential quality degradation during storage Overall, adverse events from transfusions and in effect add more to the cost of each transfusion than acquisition and procedure costs combined. While some complication risks depend on patient status or specific transfusion quantity involved, a baseline risk of complications simply increases in direct proportion to the frequency and volume of transfusion (Carson *et al.*, 2010).

1.2.7.1.1 Immunologic complications:

1-Acute hemolytic reactions occur with transfusion of red blood cells, and occur in about 0.016 percent of transfusions, with about 0.003 percent being fatal. This is due to destruction of donor erythrocytes by preformed recipient antibodies. Most often this occurs due to clerical errors or improper typing and cross matching. Symptoms include fever, chills, chest pain, back pain, hemorrhage, increased heart rate, shortness of breath, and rapid drop in blood pressure (Carson *et al*., 2010).

2-Delayed hemolytic reactions occur more frequently (about 0.025 percent of transfusions) and are due to the same mechanism as in acute hemolytic

reactions. However, the consequences are generally mild and a great proportion of patients may not have symptoms (Carson *et al.*, 2010).

3-Febrile nonhemolytic reactions are due to recipient antibodies to donor white blood cells, and occur in about 7% of transfusions. This may occur after exposure from previous transfusions. Fever is generally short lived and is treated with antipyretics, and transfusions may be finished as long as an acute hemolytic reaction is excluded (Carson *et al.*, 2010).

4-Allergic reactions may occur when the recipient has preformed antibodies to certain chemicals in the donor blood, and does not require prior exposure to transfusions (Carson *et al.*, 2010).

5-Posttransfusion purpura is a rare complication that occurs after transfusion containing platelets that express a surface protein HPA-1a. Recipients who lack this protein develop sensitization to this protein from prior transfusions, and develop thrombocytopenia about 7–10 days after subsequent transfusions. Treatment is with intravenous immunoglobulin, and recipients should only receive future transfusions with washed cells or HPA-1a negative cells (Carson *et al.*, 2010).

6-Transfusion-associated acute lung injury (TRALI) is an increasingly recognized adverse event associated with blood transfusion. TRALI is a syndrome of acute respiratory distress, often associated with fever, non-cardiogenic pulmonary edema, and hypotension, which may occur as often as 1 in 2000 transfusions (Carson *et al.*, 2010).

1.2.7.1.2 Non immunologic complications:

1-Rarely, blood products are contaminated with bacteria. This can result in life-threatening infection, also known as transfusion-transmitted bacterial infection. The risk of severe bacterial infection is estimated, as of 2002, at about 1 in 50,000 platelet transfusions, and 1 in 500,000 red blood cell transfusions. Contamination is also more common with longer duration of storage, especially

when exceeding 5 days. Sources of contaminants include the donor's blood, donor's skin, phlebotomist's skin, and from containers. Contaminating organisms vary greatly, and include skin flora, gut flora, or environmental organisms (Carson *et al*., 2010).

2-Since the advent of HIV testing of donor blood in the 1980s the transmission of HIV during transfusion has dropped dramatically. Prior testing of donor blood only included testing for antibodies to HIV. However, due to latent infection, (the "window period" in which an individual is infectious, but has not had time to develop antibodies), many cases of HIV seropositive blood were missed. The development of a nucleic acid test for the HIV-1 RNA has dramatically lowered the rate of donor blood seropositivity to about 1 in 3 million units (Carson *et al*., 2010).

3-The transmission of hepatitis C via transfusion currently stands at a rate of about 1 in 2 million units. As with HIV, this low rate has been attributed to the ability to screen for both antibodies as well as viral RNA nucleic acid testing in donor blood (Carson *et al*., 2010).

4-Other rare transmissible infections include hepatitis B, syphilis, Chagas disease, cytomegalovirus infections (in immunocompromised recipients), HTLV, and Babesia (Carson *et al*., 2010).

5-Hypothermia can occur with transfusions with large quantities of blood products, which normally are stored at cold temperatures. Core body temperature can go down as low as 32 °C and can produce physiologic disturbances. Prevention should be done with warming the blood to ambient temperature prior to transfusions (Carson *et al*., 2010).

6-Transfusions with large amounts of red blood cells, whether due to severe hemorrhaging and/or transfusion inefficacy (see above), can lead to an inclination for bleeding. The mechanism is thought to be due to disseminated intravascular coagulation, along with dilution of recipient platelets and

coagulation factors. Close monitoring and transfusions with platelets and plasma is indicated when necessary (Carson *et al.*, 2010).

7- Circulatory overload: The management is that of cardiac failure. These reactions are prevented by a slow transfusion of packed red cells or of the blood component required, accompanied by diuretic therapy (Hoffbrand *et al*., 2006).

8- Iron overload: Repeated red cell transfusions over many years, in the absence of blood loss, cause deposition of iron initially in reticuloendothelial tissue at the rate of 200-250 mg/unit of red cells. After 50 units in adults, lesser amounts in children, the liver, myocardium and endocrine glands are damaged with clinical consequences. This is a major problem in thalassaemia major and other severe chronic refractory anaemias (Hoffbrand *et al*., 2006).

9-Metabolic alkalosis can occur with massive blood transfusions due to the breakdown of citrate stored in blood into bicarbonate (Carson *et al*., 2010).

10-Hypocalcemia can also occur with massive blood transfusions due to the complex of citrate with serum calcium (Carson *et al*., 2010).

1.2.8 Repeated blood transfusions:

Patients who require repeat transfusion after an interval of more than 72 h must have a new sample sent before the next transfusion to detect any clinically significant antibody that may have been stimulated in an anamnestic response by the recent transfusion. Severe hemolytic transfusion reactions still occur due to failure to observe this simple rule; many could be avoided. In the case of an undetected haemolytic transfusion reaction, in addition to the antibody screen, a direct antiglobulin test (DAT) should be performed on the red cells of the new sample to detect any alloantibodies attached to donor red cells but not free in the Serum (Hoffbrand *et al*., 2005).

Multi-transfused patients defined as those patients who have received three or more blood transfusions (Coles., 1981)

1.2.9 Antibody Screening Test:

When exposed to foreign antigens, via transfusion or pregnancy, an individual may produce an antibody directed against foreign antigens. This antibody is an alloantibody and is found in the plasma. A test performed for detection of antibody in the plasma is the antibody screen test. Antigens are the most common immunogens for atypical or red cell stimulated antibodies. For antibody screen and identification tests, serum or plasma are acceptable specimens. Patient and donor plasma is screened for atypical antibodies using commercially prepared cells. They are group O cells that have been tested for the presence of the most commonly encountered antigens. The cells are provided from the manufacturer in sets of either two or three vials. Each vial contains cells from a single, unique donor. The vials are provided with a description of the antigen content of each of the cells. Testing the plasma of the patient or donor with the commercially prepared cells is the basic antibody screen procedure. Testing incorporates varying conditions of reactivity including temperature and use of potentiating media. The media of reactivity includes immediate spin saline, potentiating media such as low ionic strength substance (LISS), and anti-human globulin (AHG) sera. Results of each phase are recorded and evaluated. This is a screening test and test results indicate the presence of an antibody, but not the identity of the antibody. The use of antigrams and incorporation of knowledge of optimal temperature and media of reactivity for the potential antibodies allows the possibilities to be narrowed. For specific identification, an antibody identification panel must be utilized and results evaluated (Whitlock., 2012).

Autocontrol Antibody screen testing may include the use of an autocontrol. The autocontrol is an additional test that contains the individual's red cell suspension and plasma and is tested in parallel with the antibody screen cells. The results of the autocontrol are recorded along with the screen results. A

positive result in the autocontrol at the AHG phase indicates an antibody coating the red cells. This antibody may be removed from the cells and identified in the same manner as antibody in plasma. The use of an autocontrol is optional. Alternately, a direct antiglobulin test may be performed if the antibody screen is positive (Whitlock., 2012).

1.2.9.1 Direct Anti-globulin Test:

A test detects antibodies coating the surface of the red blood cells *in vivo*. Using a combination of different AHG sera can provide additional information related to the specificity of the substance(s) coating the red cells. Polyspecific AHG contains a mixture of anti-IgG and anti-C3. Monospecific AHG is either anti-IgG or anti-C3. Reactivity in one or more AHG sera provides specificity of the substance(s) coating the red cells (Whitlock., 2012).

1.2.10 Antibody Identification:

The presence of a positive antibody screen requires identification of the antibody. If one or more antibody screen cells present with a positive result, then an antibody identification panel is performed. If only the autocontrol is positive, a DAT is necessary. A positive DAT will require removal of the antibody by elution and testing of the eluate. An antibody identification panel is a series of 8 to 16 red cells from unique Group O donors that have been antigen typed for the common red cell antigens (Whitlock., 2012).

1.2.11 Additional Antibody Identification Tests:

When results of the initial antibody identification are not definitive, phases and temperatures of reactivity and red cell phenotyping results are considered. When all of these factors have been assessed and a definitive result has not been obtained (Whitlock., 2012).

1.2.11.1 Enzyme Testing:

Enzymes are used in blood bank testing to aid in identification of antibodies. Proteolytic enzymes, such as ficin and papain, affect antigen-antibody reactions by either enhancing (strengthening) or inhibiting (eliminating) the reactivity. Enhancement makes antigens more accessible. Destruction of antigens on the surface of the cells by the enzyme creates inhibition of some reactions, because enzymes inhibit the reactions of some antibodies, inclusion in the identification process may rule out additional antibodies in the plasma (Whitlock., 2012).

1.2.12 blood transfusion in cancer patients:

With specific cancer treatment the blood product used as general supportive therapy (Hoffbrand *et al*., 2006).

1.2.13 Tumor:

Is defined as the abnormal mass of tissue (new growth) and is characterized by unceasing abnormal and excessive proliferation of cells (Danish., 2009).

1.2.13.1 Types of Tumor

1.2.13.1.1 Benign tumors:

It is the tumor characteristics of which are relatively innocent, such as:

- It will remain localized.
- It cannot spread to the other sites.
-Amenable to local surgical removal.
-Patient survives (Danish., 2009).

1.2.13.1.2 Malignant tumor (Cancer):

The cancer is destructive an dangerous, have characteristics such as:

-It can invade and destroy adjacent structures.
-It spreads to distant sites (metastasis).
- Patient dies (Danish., 2009).

1.2.14 Previous studies:

Previous study showed red cell transfusions are a valuable health care resource especially for thalassemics, patients of myeloproliferative disorders, hematological disorders, end stage renal failure, patients of leukemia and organ transplant patients. Chronic red cell transfusions can cause unwanted complications called transfusion reactions in a patient. Development of alloantibodies to red cell antigens is an important immune mediated delayed hemolytic transfusion reaction. It is a matter of great concern in multi-transfused patients and in patients who have had multiple pregnancies. Alloimmunization results from disparity between the donor and patient antigens. Prior exposure to donor antigens can lead to anamnestic or secondary response where even very small amounts of donor antigenic RBCs can elicit an alloimmune response resulting in increase in antibody production leading to red cell destruction since the patient is already immunized Alloimmunization occurs when incompatible antigens introduced in an immune-competent host evoke an immune response leading to irregular antibody formation. Alloimmunization against RBCs can result in delayed hemolytic transfusion reactions which can range from destruction of RBCs within hours or even minutes to decrease of survival by a few days or can cause hemolytic disease in newborns. Development of alloantibodies thus complicates and limits transfusion therapy, contributing not only to technical complications but also to morbidity and mortality. (Sood *et al*., 2013)

In another study, the overall frequency of alloantibody formation was found to be 22.06% in 68 multi-transfused patients the most frequently detected alloantibodies were anti-K, anti-E, and anti-C (Obeidi N *et al*., 2010).

Reyhaneh and colleagues (2013) showed alloimmunization is a common problem in patients undergoing blood transfusion. Most studies have been carried out on patients who chronically receive blood transfusions. In patients

affected with haemoglobinopathies, haematologic diseases, various types of cancer, recipients of organ transplantation, and patients with renal failure, the prevalence of alloimmunization has been reported to be up to 60 per cent, while in hospitalized patients receiving transfusions alloimmunization has been seen in about 1 to 10 per cent. Along with alloantibodies RBC autoantibodies are also developed. The rate of RBC alloimmunization depends on the characteristics of the population being studied. The differences among the various populations, including race and sex differences make generalization of the results difficult. The frequency of blood group antigens is known among Iranian population, but so far the relative frequencies of RBC alloantibodies in a general population of alloimmunized patients who occasionally receive blood transfusion, have not been determined. The current study was designed to assess the frequency of alloimmunization to RBC antigens in the occasionally transfused patients due for surgery in Iran. (Reyhaneh *et al*., 2013)

Patel and colleagues (2009) multi-transfused patients suffering from β-thalassemia, sickle cell disease (SCD), chronic renal failure (CRF), etc. need frequent blood transfusions thus facing a constant risk of alloimmunization , Present study was undertaken in selected groups of multi-transfused patients to evaluate their ability to generate blood group antibodies (Patel *et al.*, 2009).

Al-Joudi and colleagues (2011) showed the association between the development of alloantibodies with blood group A was not clear (Al-Joudi *et al.*, 2011). Vichinsky and colleagues (1990) studied alloimmunization among 80 transfusion patients. Alloimmunization was found in 3 cases and belonged mainly to the Rh system and the relation between the numbers of blood units transfused and antibody formation was insignificant, but it was an important factor for increased alloimmunization in patients who receive multiple transfusions (Vichinsky *et al*., 1990).

1.3Rationale:

Cancer patients need blood transfusions because of the cancer itself: some cancers cause internal bleeding, which can lead to anemia, cancers that start in the bone marrow (such as leukemias) or cancers that spread there from other places may crowd out normal blood-making cells leading to low blood counts, cancer patients may develop anemia of chronic disease, this anemia results from certain long-term medical conditions that affect the production and lifespan of red blood cells, and cancer can also lower blood counts by affecting organs such as the kidneys and spleen, which help keep enough cells in the blood.

Cancer treatments also lead to the need for blood transfusions: surgery to treat cancer may lead to blood loss and a need for red blood cell or platelet transfusions, most chemotherapy drugs affect cells in the bone marrow, this commonly leads to low blood cell counts, and can sometimes put a person at risk for life-threatening infections or bleeding, when radiation is used to treat a large area of the bones, it can affect the bone marrow and lead to low blood cell counts, and bone marrow transplant (BMT) or peripheral blood stem cell transplant (PBSCT) patients get large doses of chemotherapy and/or radiation therapy, this destroys the blood-making cells in the bone marrow. These patients often have very low blood cell counts after the procedure and need transfusions.

In contrast many immunologic reactions were occur, the Frequency of anti-K, anti-Fya, etc. has increased, evidently due to the increased number of Multi-transfused patients and the absence of measures for preventing immunization to antigens other than D. In many cancer patients irregular blood group antibodies, originated from multi-transfusion may cause severe hemolytic transfusion reactions, and still occur due to failure to observe this simple rule and many could be avoided, so as to identify the most frequent circulate antibodies among Sudanese multi-transfused cancer patients attending RICK. There is few previous study have been done in Sudan.

1.4 Objectives:

1.4.1 General objective:

To detect the frequency and specificity of alloantibodies against RBCs antigens among Sudanese cancer patients with multi-blood transfusion in Khartoum state during the period from February 2014 to April 2014.

1.4.2 Specific objectives:

1-To screen the presence and identify type of alloantibodies in sudanese cancer patients with multi-blood transfusion.

2-To associate between number of repeated transfusion and alloimmunization.

3-To detect the relationship between alloimmunization and ABO and Rh blood grouping.

5-To determine the association between alloantibodies and age and sex.

6- To determine the association between alloantibodies and type of diseases.

Chapter Tow

Materials and methods:

2.1 Study design:

This study was descriptive cross-sectional study of 203 multi-transfused cancer patients in Khartoum state from February up to April 2014. Samples collection and data of multi-transfused were done In Radiation and Isotope Center-Khartoum. Laboratory investigation was done in Research Laboratory, College of Medical Laboratories of Sudan University of science and Technology.

2.2 Sample size:

Two hundreds and three cancer patient multi-transfused were randomly selected for this study.

2.3 Study population:

Multi-transfused Sudanese cancer patients attending to Radiation and Isotope Center-Khartoum were been included in this study.

2.4 Inclusion criteria:

Multi-transfused Sudanese cancer patients with whole blood.

2.5 Exclusion criteria:

Patients with autoimmune disease (positive for DAT) or any medical condition, which interfere with detection of antibody.

2.6 Method of data collection:

Data had been collected by using structural interviewing questionnaire, data include of age, gender, diagnosis, number of blood transfused and if there any previous hemolytic transfusion ration.

2.7 Ethical consideration:

This study was carried after approval had been taken from the Sudan University of science and technology. Verbal consent was taken from multi-transfused cancer patients under study.

2.8 Sampling:

Samples had been collected by a septic technique. 2.5 ml of blood from multi-transfused cancer patients was drawn with EDTA anticoagulant. Samples also had been centrifuged at 3000 rpm for 5 minutes and separated serum was tested.

2.9 Laboratory protocol:

Samples were grouped then were tested for antibody screening and antibody identification using commercially prepared cells, and specific an antibody identification panel was utilized and results were obtained by indirect method of anti-human globulin testing (IDT).

2.10 Methods:

2.10.1 ABO and Rh blood grouping:

1. Typing for ABO and Rh Antigens: Commercial antisera were combined with red cells from the patient.

2. Typing for Antigens of Other Blood Group Systems: Commercial antisera were combined with red cells from the recipient (Whitlook 2012).

2.10.2 Indirect Antiglobulin Testing:

Principle**:** The principle of anti-human globulin testing usually combines a known antigen or antibody with either plasma or cells that have an unknown component. The test must include a $37C^{o}$ incubation phase. During this phase, immunoglobulin G (IgG) molecules sensitize antigen-carrying RBCs. Enhancement media may be added to increase the degree of sensitization. Tubes might be centrifuged and observed for hemolysis or agglutination following the incubation. To observe for agglutination, the tube is gently tilted or rolled to dislodge the cell button. The degree of reactivity is graded as 0 (negative, no agglutination present) to weak reaction + (barely visible to the naked eye) to 4+ (one solid agglutinate) Negative results in the incubated tubes require that check cells be added as a form of quality control. After adding a

drop of coated cells to each negative tube, the tubes are centrifuged and examined. Agglutination should be seen (Whitlook 2012).

2.10.2.1 Materials:

Micropipette, Disposable pipette tips, Centrifuge, 12×57mm test tubes, Reagent red for screening, identification of unexpected antibodies, Incubator 37℃, Coomb's reagent and its control, SSA as washing control, Normal saline (NS), Disposable slide sand Microscope (Whitlook 2012).

2.10.2.2 Sample: In this study plasma was used.

2.10.2.3 Procedure:

1- Specimens were centrifuged at 5000 rpm for 3 minutes. (Speed and time as recommended by manufacturer's directions).

2- Specimens were checked after centrifuging (e.g. hemolysis).

3- Patient information was ensuring on the sample corresponds with the patient information on the worksheet.

4- Three tubes were labelled with the patient identifier and the reagent red cells (I II III).

5- 2-3 drops of patient plasma were added to be tested to each labeled tube.

6- One drop of thoroughly mixed reagent red cells was added to the appropriate labelled tube.

7- Mixing the contents of each tube and examining all tubes for appearance and volume were done.

8- Checking and recording the temperature of the waterbath/heating block were done.

9- All tubes at 37ºC (±1 ºC) for 30-60 minutes were incubated.

10- The tubes were removed from the waterbath after incubation, then were centrifuged at 5000 rpm for 3 minutes. (Speed and time as recommended by manufacturer's directions).

11- The cells by gentle agitation were resuspended and examined macroscopically for agglutination.

12- Grade and record the 37 ºC results were done.

13- Tubes were washed a minimum of 3 times with saline under washing control, and completely decanted saline after final washing to obtain a “dry” red cell button.

14- Two drops of AHG to each tube were added.

15- Tubes were centrifuged. (Speed and time as recommended by manufacturer’s directions).

16- The cells were immediately resuspended by gentle agitation, examined macroscopically for agglutination. If the tubes were appear negative macroscopically, immediately were read microscopically.

17- Grade and record results were done.

18- The validity of negative results was confirmed by adding 1 drop of IgG sensitized cells to each tube.

19- At least grade 2 reactions were expected following the addition of IgG sensitized cells. If agglutination following the addition of IgG sensitized cells was weaker than expected or not detected, the test was invalid and must be repeated and the reason for the unexpected weak reaction identified.

20- Interpret and report antibody screen results were done. Agglutination or hemolysis was a positive test result and indicates the presence of clinically significant antibodies.

21-Report the antibody screen as positive, and identification had been done as same screening procedure (Whitlook 2012).

2.11 Statistic methods:

Collected data and tests results had been analyzed using the computer program SPSS 11.5 (statistical package of social sciences).

Value of $p < 0.05$ was considered significant. Descriptive statistics for variables of age, sex, type of cancer, ABO and Rh grouping, number of blood transfusion, an antibody screening and identification had been performed.

Chapter Three

Results

3.1 Demographic data:

Two hundreds and three multi-transfused cancer patients were analyzed according to following:

Sex; the study population was 80 males (39%) and 123 females (61%), as seen in table (3.1).

Age; the study population was 10-100 years and grouped to 10-20 years which were (15%), 21-30 (9%) , 31-40 (18%), 41-50 (20%), 51-60 (15%), 61-70 (10%), 71-80 (9%) ,81-90 (3%) and 91-100 (1%) as seen in table (3.1).

Type of cancer; the study population was classified into leukemia (40%), breast cancer (32%), ovarian cancer (10%), lung cancer (1.5%), esophageal cancer (5%), brain cancer (0.5%), liver cancer (4%), pancreatic cancer (2.5%), cervical cancer (1.5%), colon cancer (1%) and prostate cancer (2%) as seen in table (3.2).

Number of blood transfusion; the study population was grouped into 3-5 times which were (43%), 6-10 times (30%), 11-15 times (20%) and more than 15 (7%) as see in table (3.3).

3.2 Laboratory data:

This study showed that according to antibodies screening, they were 59 positive (29%) and 144 negative (71%) as seen in figure (3.1).

ABO and Rh blood group system showed A Rh positive which were (23%), B Rh positive (22%), O Rh positive (38%), AB Rh positive (7%), A Rh negative (3%), B Rh negative (1%), O Rh negative (4%), and AB Rh negative (2%) as seen in figure (3.2).

Alloantibodies positive cases showed A Rh positive which were (27%), B positive (25%), O Rh positive (31%), AB Rh positive (3%), A Rh negative

(1%), B Rh negative (1%),AB Rh negative (3%) O Rh negative (7%) as seen in figure (3.3).

The relationship between ABO and Rh (D) blood group system and alloimmunization antibodies was tested and observed (p value = 0.77), and there was insignificant (p value > 0.05).

According to sex, the positive distribution for antibodies screening were 27 males (46%), and 32 females (54%) as seen in figure (3.4), and relationship between sex and alloimmunization antibodies was tested and observed (p value = 0.46), and there was insignificant (p value > 0.05).

The positive cases according to age showed 10-20 years which were (17%),21-30 (3%) and 31-40 (22%), 41-50 (17%), 51-60 (14%), 61-70 (14%), 71-80 (8%), 81-90 (3%) and 91-100 (2%) as seen in figure (3.5), and relationship between age (grouped) and alloimmunization antibodies was tested and observed (p value = 0.48), and there was insignificant (p value > 0.05).

The positive cases according to type of cancer showed leukemia in 24 patients (41%), breast cancer in 17 patients (29%), ovarian cancer in 3 patients (5%), lung cancer in 3 patients (5%), esophageal cancer in 3 patients (5%), brain cancer in 1 patient (2%), liver cancer in 2 patients (3%), pancreatic cancer in 3 patients (5%), cervical cancer in 1 patient (2%) and prostate cancer in 2 patients (3%) as seen in figure (3.6),and relationship between type of cancer and alloimmunization antibodies was tested and observed (p value = 0.97), and there was insignificant (p value > 0.05).

The positive cases according to number of blood transfused showed 3-5 times which were (41%), 6-10 times (27%), 11-15 (22%) and more than 15 (10%) as see in figure (3.7), and relationship between number of blood transfused and alloimmunization antibodies was tested and observed (p value = 0.71), and there was insignificant (p value > 0.05).

The antibodies identification for positive antibody screening showed 26 anti-Kell which were (44%), 3 anti-s (5%), 2 anti-C^w (3%), 2 anti-E (3%), 4 anti-e (7%), 8 anti-Le^b (14%), 4 anti-Kidd (7%), 3 anti-c (5%), 1 anti-V (2%), 3 anti-$duffy^a$ (5%) and 3 anti-N (5%) as in figure (3.8).

3.1 Demographic data:

Table (3.1) Distribution of study population according to sex and age:

Variables		Number	Percentage
Sex	Male	80	39%
	Female	123	61%
Age/year	10-20	30	15%
	21-30	19	9%
	31-40	35	18%
	41-50	40	20%
	51-60	31	15%
	61-7	21	10%
	71-80	18	9%
	81-90	7	3%
	91-100	2	1%

Table (3.2) Frequency of types of cancer in study population:

Variables		Number	Percentage
Type of cancer	Leukemia	82	40%
	Breast cancer	65	32%
	Ovarian Cancer	19	10%
	Esophageal Cancer	10	5%
	Liver Cancer	9	4%
	Pancreatic Cancer	5	2.5%
	Prostate Cancer	4	2%
	Cervical Cancer	3	1.5%
	Lung Cancer	3	1.5%
	Colon Cancer	2	1%
	Brain Cancer	1	0.5%

Table (3.3) Frequency of blood transfusion in study population:

Number of blood transfusion	Number	Percentage
3-5 times	88	43%
6-10 times	61	30%
11-15 times	41	20%
More than 15 times	13	7%

3.2 Laboratory data:

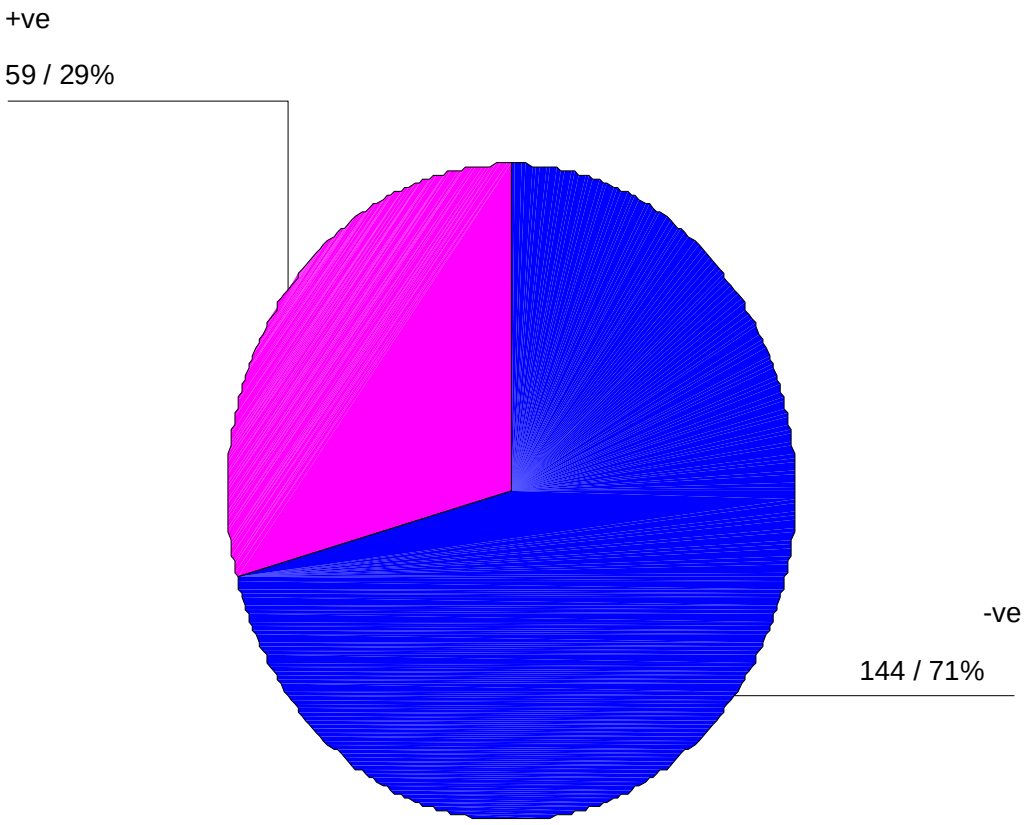

Figure (3.1): Percentage of antibodies screening in study population.

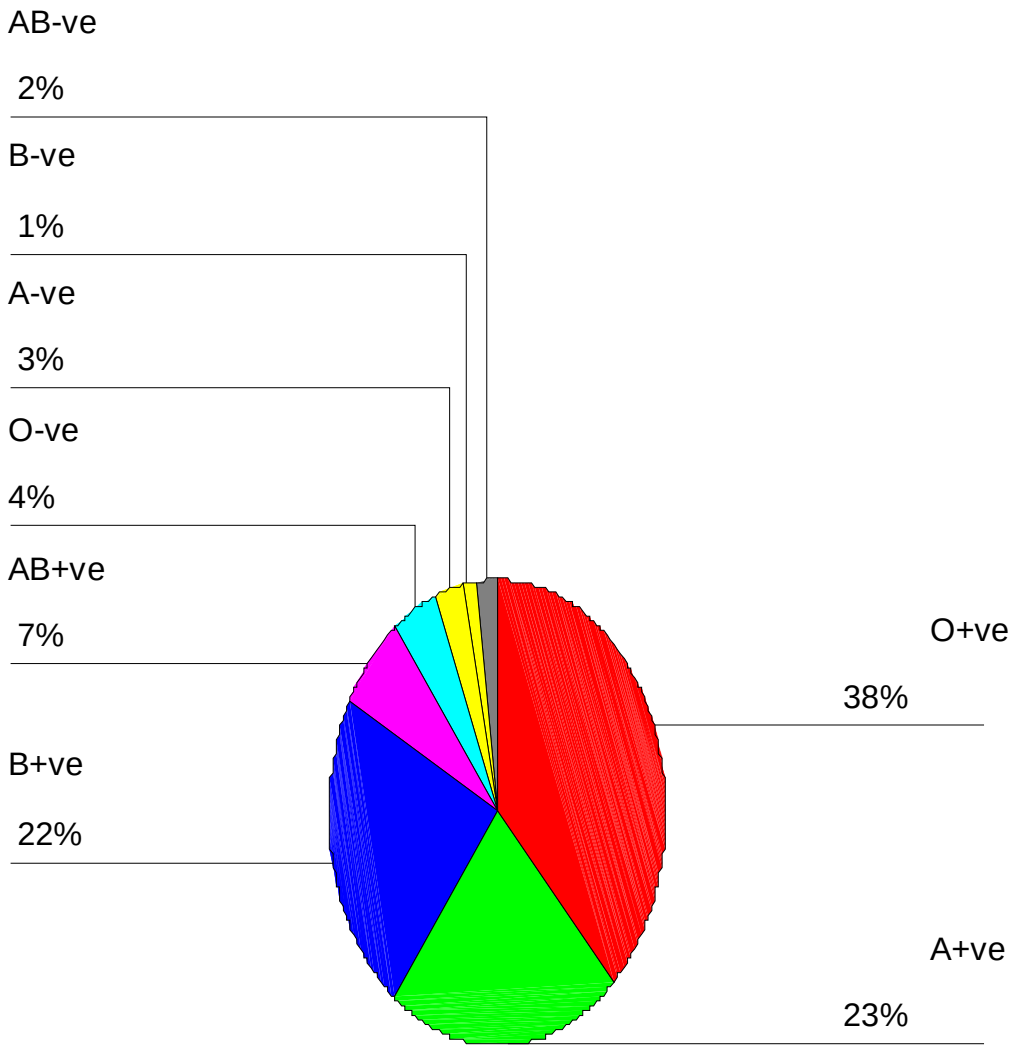

Figure (3.2): Percentage of ABO and Rh blood group system in study population.

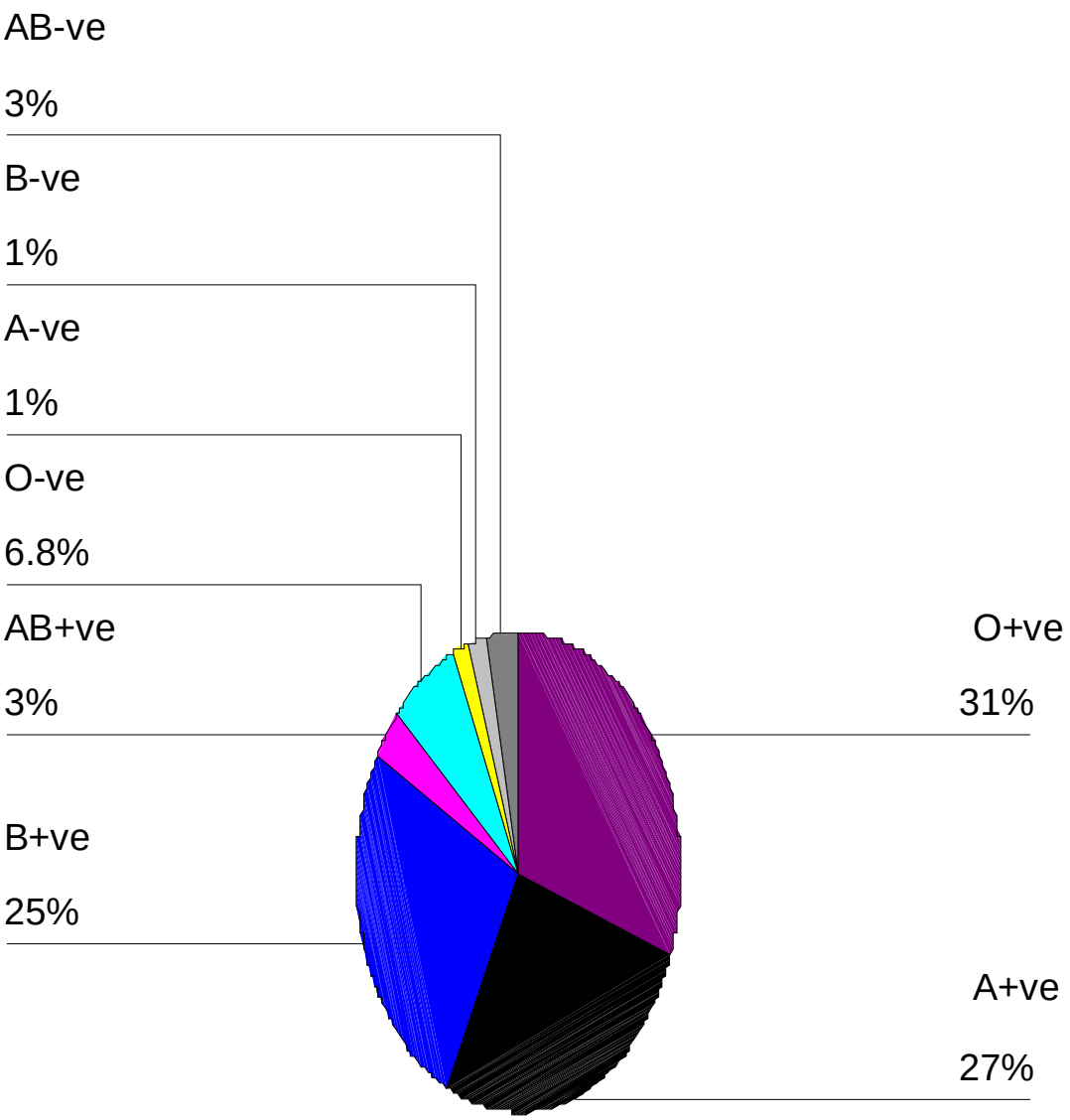

Figure (3.3): Percentage of ABO and Rh blood group system in alloantibodies positive cases.

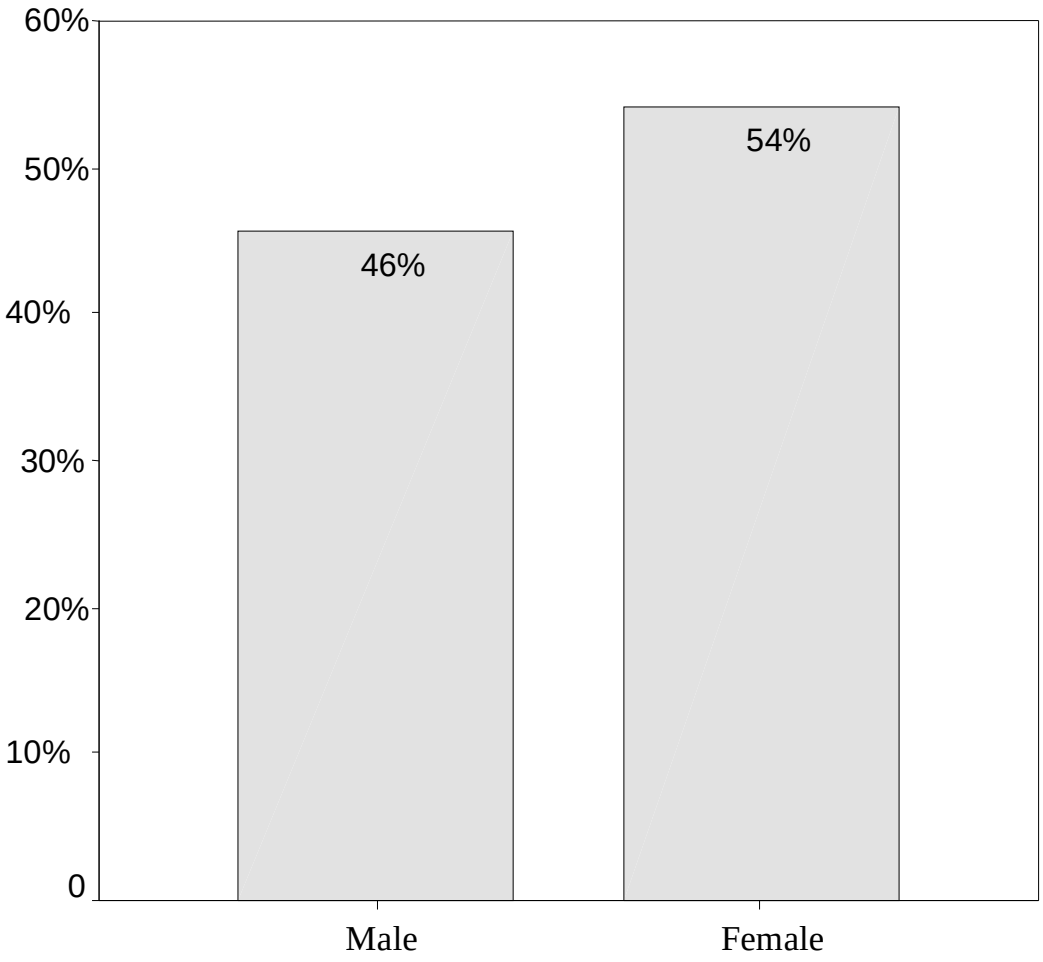

Figure (3.4): Distribution of alloantibodies positive cases according to sex.

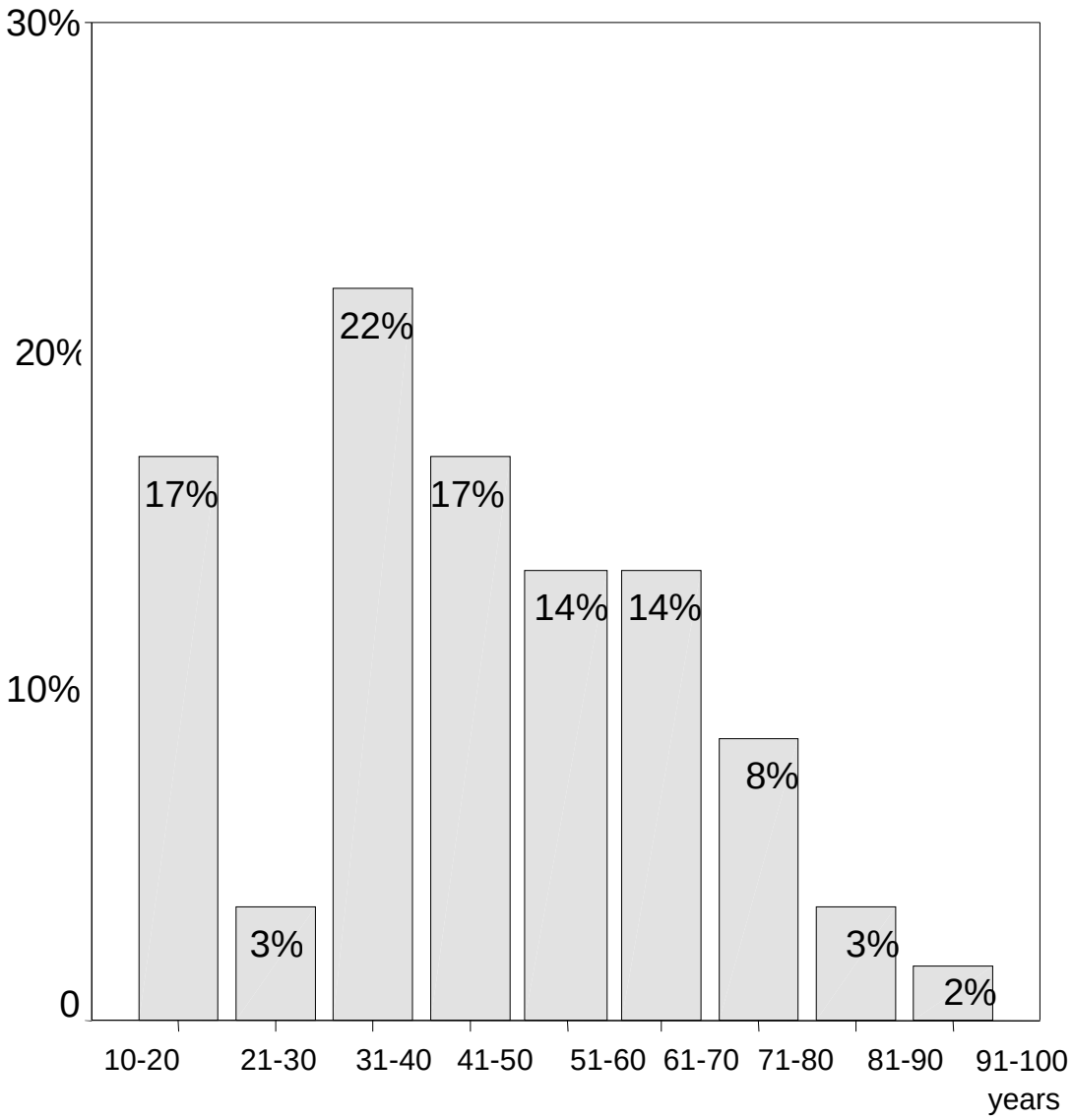

Figure (3.5): Frequency of alloantibodies positive cases according to age.

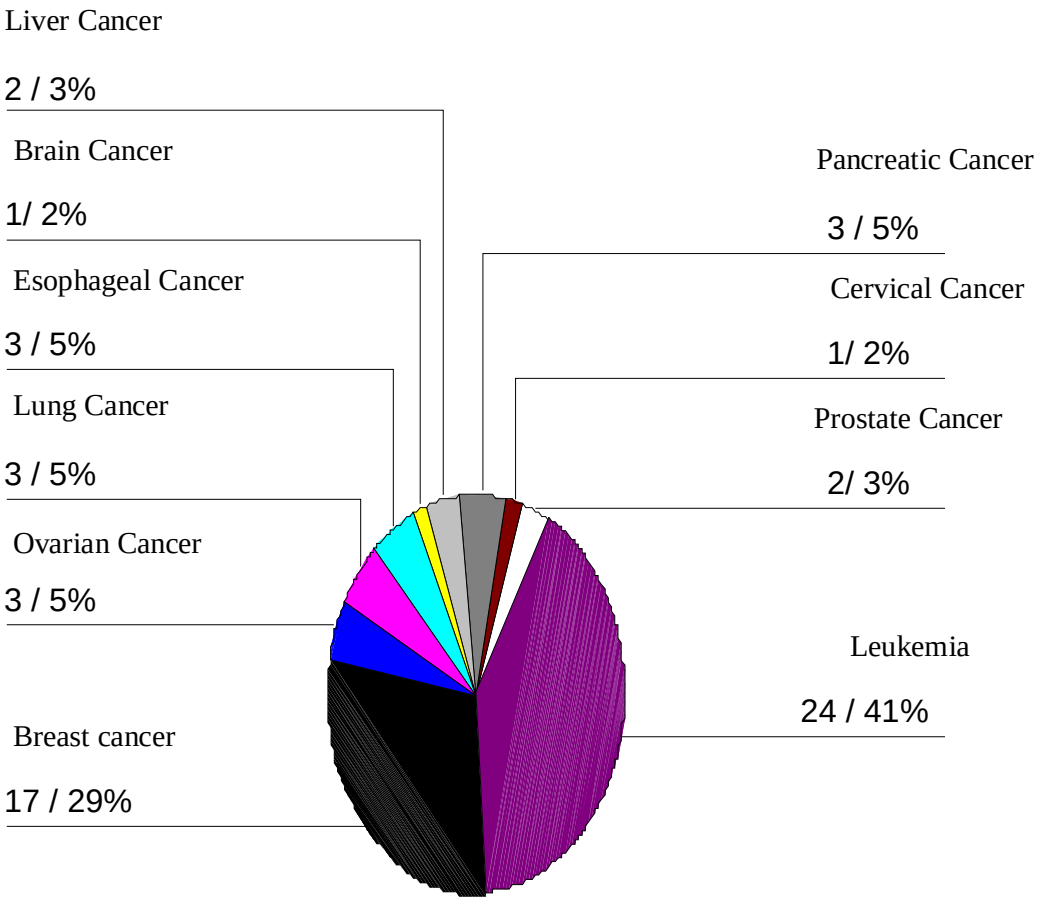

Figure (3.6): Distribution of alloantibodies positive cases according to type of cancer.

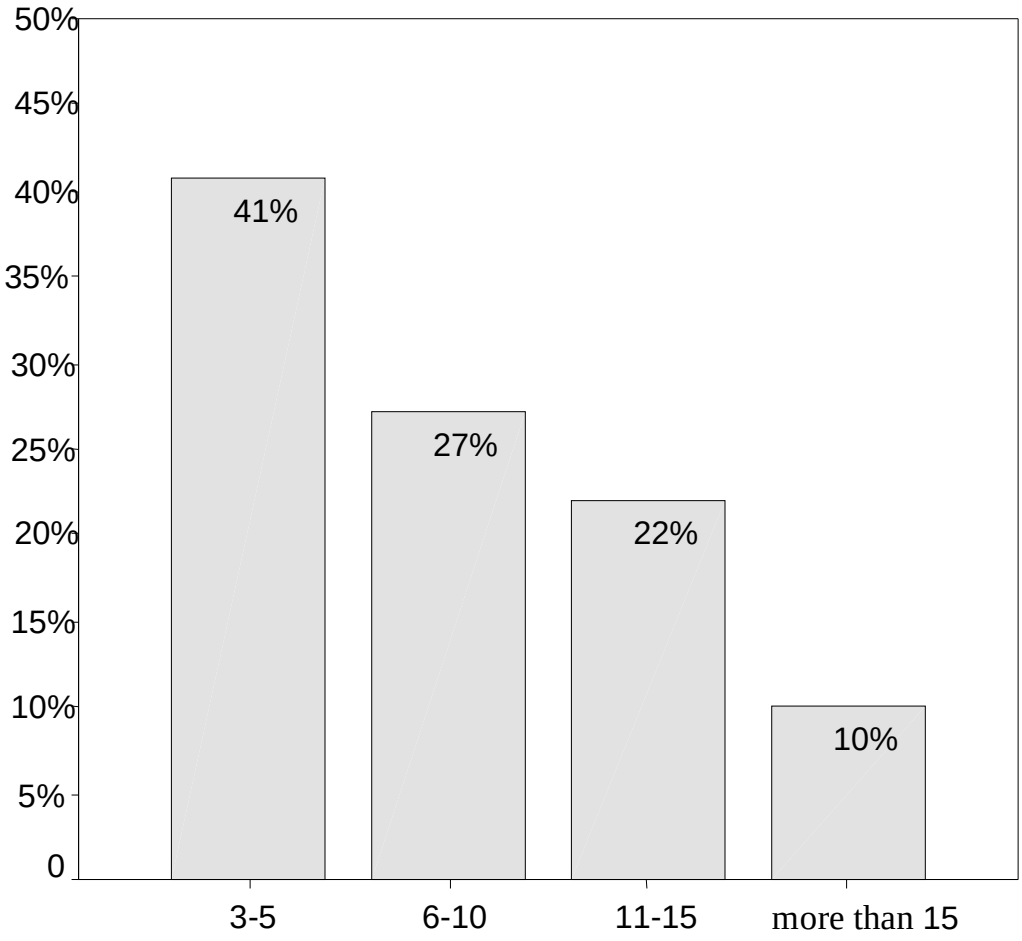

Figure (3.7): Percentage of numbers of blood transfusion in alloantibodies positive cases.

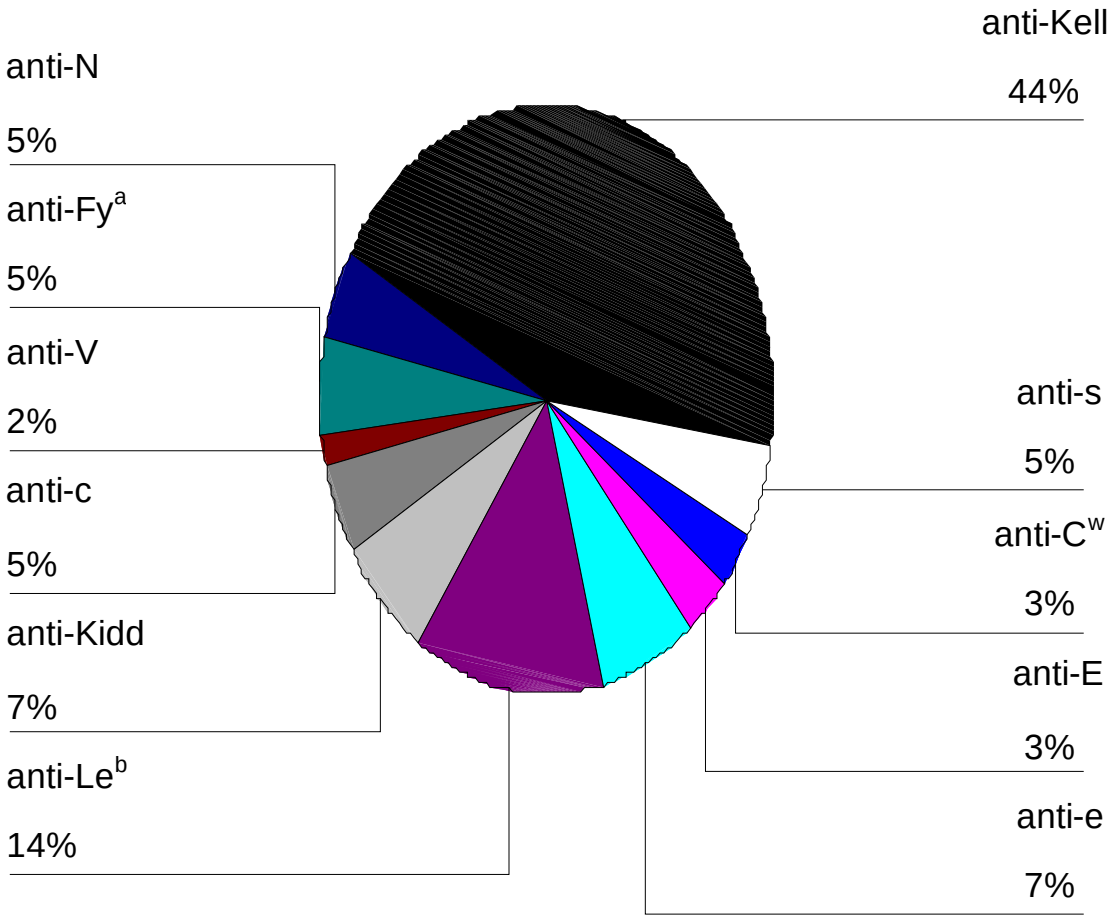

Figure (3.8): Distribution of alloantibodies positive cases according to antibodies identification

Chapter Four

Discussion, conclusion and recommendations:

4.1 Discussion:

The frequencies of alloantibodies against one of the major blood group system (rhesus ((Rh)) and some of minor blood groups system (Kell, Kidd, Duffy, Lewis, P and MNSs) were studied in 203 multi-transfused patient, they were grouped for ABO and Rh (D). An antibody screening was performed for multi-transfused patients, irrespective of whether they were Rh- D positive or negative ,in this study 169 multi-transfused patients were Rh(D) were positive (83%) and 34 were Rh(D) negative (17%). Antibodies were detected in 59 multi-transfused patients (29%), only 8 of them were Rh (D) negative and anti-D was not detected when they screened.

Antibody identification was performed for positive cases, identified antibodies showed that the most common frequent antibody was anti-Kell followed by anti-Le^b, anti-Kidd, anti-e, anti-s, anti-c, anti-Fy^a, anti-N, anti-C^w, anti-E and anti-V, and this result was agreed with study of Obeidi who found that anti-Kell was the most frequent antibody (Obeidi N *et al*., 2010).

The results of this study reveals that insignificant relationship was found between alloimmunization and sex with p value (0.46), and this was agreed with study of Sood and colleagues who found that there was no significant association between alloimmunization and gender.

The results of this study reveals that insignificant relationship was found between alloimmunization age and type of cancer with p value (0.48, 0.97) respectively, and this was agreed with study of (Sood *et al*., 2013) who reported that no significant relationship between a diagnosis and alloimmunization in transfusion patients.

An insignificant relationship was observed between alloimmunization and ABO-Rh blood group system with p value (0.77), and this was agreed with

study of Al-Joudi who found that there was no significant association between alloimmunization and ABO and Rh (D) blood group (Al-Joudi *et al.*, 2011). An insignificant relationship was observed between alloimmunization and number of blood transfusion with p value (0.71), and this was agreed with study of Vichinsky who found that the relation between the numbers of blood units transfused and antibody formation was insignificant, but the number of blood transfusion is an important factor for increased alloimmunization in patients who receive multiple transfusions (Vichinsky *et al.*, 1990).

4.2 Conclusion:

1-Red blood cells alloantibodies were found in 29% of multi-transfused cancer patients.

2-Most common identified antibodies were anti-Kell followed by anti-Le^b, anti-Kidd, anti-e, anti-s, anti-c, anti-Fy^a, anti-N, anti-C^w, anti-E and anti-V.

3-There was no relationship between alloimmunization and sex.

4-Tere was no relationship between alloimmunization and age.

5-There was no relationship between alloimmunization and type of cancer.

6-There was no relationship between alloimmunization and number of blood transfusion, ABO and Rh blood group system.

4.3 Recommendations:

1-Further studies using lager sample size needed for more accurate result.

2-All multi-transfused cancer patients ABO and D Rh should be screened for presence of red blood cells alloantibodies.

3-The screening cells and methods used in red cells antibody screening should be complied with the guidelines for compatibility procedures in blood transfusion laboratories.

4-Positive antibody screening multi-transfused cancer patients should be phenotyped for easier alloantibody identification.

5- The Kell blood group system should be screened in all blood transfusion laboratories.

6-Other technique rather than coomb's technique must used for antibody screening and identification of other uncommon and unidentified red cell alloantibodies.

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Appendices

Appendix (1)

Sudan University of science and technology

College of graduate studies

Questionnaire

Determination of Red Cell Alloimmunization among Sudanese Cancer Patients with Multi-blood Transfusion - Khartoum State

Ethic information: I agree…( ) I don't agree…( )

General information:

Serial No……..

Name……..….
Age…….……sex…….

Diagnosis……

Blood transfusion history:

Number of blood transfused

3-5 ( ) 6 to 10 ( ) 11 to 15 ( ) more than 15 ( )

Is there any hemolytic transfusion reaction?

Yes ( ) number of hemolytic transfusion ( ) No ( )

Serological investigation result:

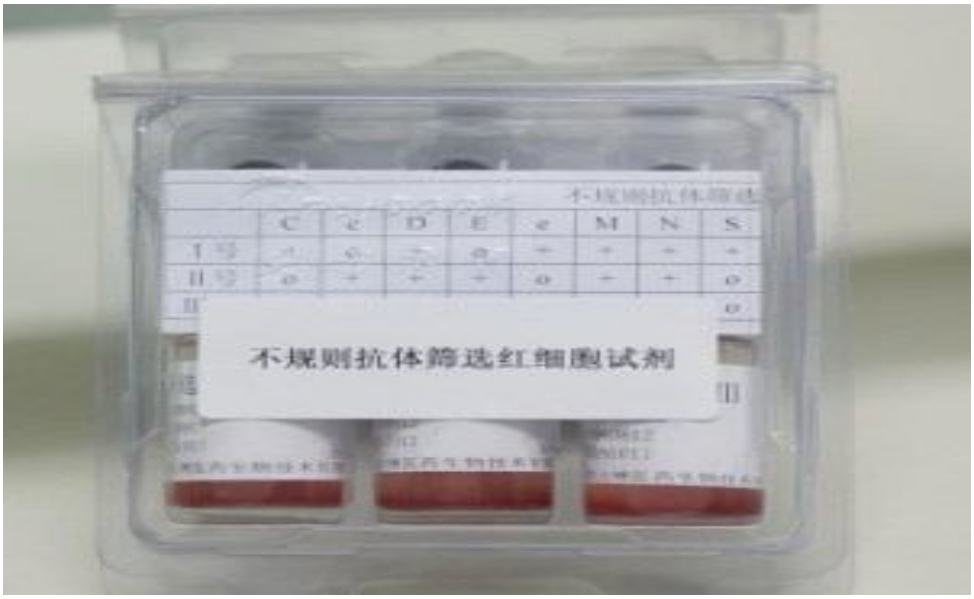
ABO group …….

Rh (D) ……..……..

Antibody screening……

Antibody identification……

Appendix (2): Reagent of screening cells:



SCREENCELL I-II-III are specifically selected cells from 3 blood group O donors (no pools) in optimal antigen presentation for the screening of the most frequent clinical relevant antibodies. Each vial contain 10 ml of red blood cells in 2-4% suspension in a buffered with special preservatives medium that contains chloramphenicol (0.25mg/ml) , neomycin (0.1mg/ml) and gentamycin sulfate (0.05mg/ml), Store at 1-10℃.. The suitable antigen profile sheet is included with every panel lot. SCREENCELL reagent red cells have been designed for AHG (combs') reagent.

Appendix (3): Reagent of identification cells (panel cells):

PANOCELL-10 indents 11 are selected panel of 11 cells from 11 blood groups O donors in optimal antigen presentation for identification of most frequent clinical relevant antibodies. Each contain 3 ml vial of red blood cells, in 2-4% suspension in a buffered with special preservatives medium that contains chloramphenicol (0.25mg/ml) , neomycin (0.1mg/ml) and gentamycin sulfate (0.05mg/ml), Store at 1-10℃. The suitable antigen profile sheet is included with every panel lot. PANOCELL-10 reagent red cells have been designed for AHG (combs') reagent.