INTRODUCTION

Thalassemia is a group of anemias that results from inherited defects in the synthesis of hemoglobin. Thalassemia is among the most common genetic disorders worldwide, occurring more frequently in the Mediterranean region,[1] the Indian subcontinent, south east Asia, and west Africa.[2] In Egypt, beta (β) thalassemia major represents a major public health problem. The carrier rate varies between 5.5 and 9%; it was estimated that 1:1500 live births per year have β-thalassemia.[3]

Pathophysiology of Thalassemia

In adults, the predominant hemoglobin (Hb) is HbA which consists of four chains: two alpha (α) and two beta (β) chains (figure 1). In thalassemia, the synthesis of either the α or β chains is reduced or absent, thus preventing the formation of a normal Hb molecule. In α-thalassemia, the α-chain is reduced or absent, whereas in β-thalassemia the β-chain is reduced or absent. The excess globin chains denature in the red cell precursors and cause premature cell death. The two forms of thalassemia (α or β) differ in their clinical presentation and severity. The α-thalassemias are more frequent in African population, Mediterranean, or Asian descent, whereas the β- thalassemias are more frequent in persons from south east Asia.[4]

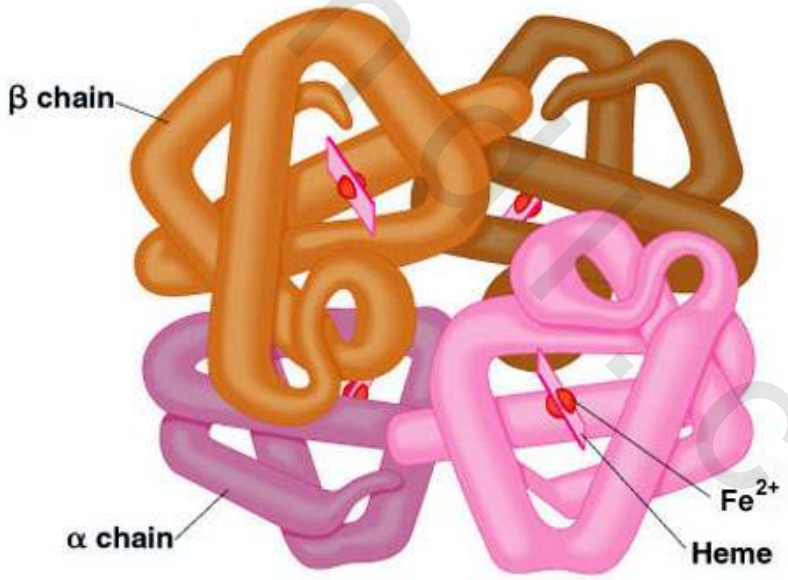

Figure 1: Structure of hemoglobin.[5]

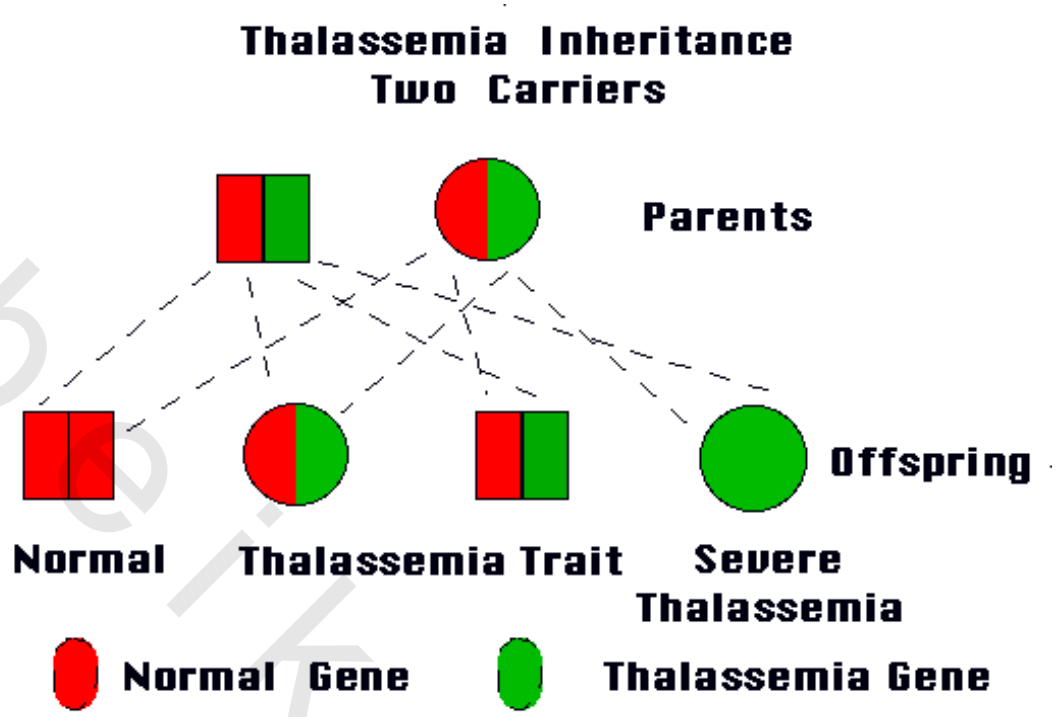

Figure 2: Thalassemia inheritance.[6]

Clinical Types of β -Thalassemia: (Figure 2)

1- Beta-Thalassemia Minor (Trait):

Carriers of thalassemia minor are usually clinically asymptomatic but sometimes have mild anemia. The characteristic hematological features are microcytosis, hypochromia and increased HbA_2 levels.[7]

2- Thalassemia Intermedia:

It is a type of thalassemia in which the clinical severity is somewhere between the mild symptoms of β thalassemia trait and the severe manifestations of β thalassemia major. The diagnosis is a clinical one that is based on the patient maintaining a satisfactory Hb level between 8 -10 g/dL at the time of diagnosis without the need for regular blood transfusions.[8]

Individuals with thalassemia intermedia present later than thalassemia major and have milder anemia. Hyperplasia of erythroid marrow with the possibility of extramedullary erythropoiesis, a compensatory mechanism of bone marrow (BM) to overcome chronic anemia, is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spines. Enlargement of the spleen is also a consequence of its major role in clearing damaged red cells from the blood stream.[9]

Extramedullary erythropoiesis may cause neurological problems such as spinal cord compression with paraplegia and intrathoracic masses. As a result of ineffective erythropoiesis and peripheral hemolysis, thalassemia intermedia patients may develop gall stones, which occur more commonly than in thalassemia major. Patients with thalassemia intermedia frequently develop leg ulcers and have an increased predisposition to thrombosis as compared to thalassemia major, especially if splenectomised. Such events include deep vein thrombosis (DVT), portal vein thrombosis, stroke and pulmonary embolism.[10]

3- Beta Thalassemia Major:

Two main variants of β-thalassemia exist: β^0 and β^+. In the β^0 form, no β chains are produced, and in the β^+ form, a reduced amount of β chains is produced. The molecular pathogenesis of β-thalassemia is heterogeneous. Different mutations in the β globin gene have been described. Most lesions are point mutations, and some involve small deletions.[11]

The reduced amount (β^+) or absence (β^0) of β globin chains results in a relative excess of unbound α globin chains that precipitate in erythroid precursors in the BM, leading to their premature death and hence to ineffective erythropoiesis.[12]

Peripheral hemolysis occurs when insoluble α globin chains induce membrane damage to the peripheral erythrocytes.[13] Anemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the BM (up 25 to 30 times the normal), which in turn causes bone deformities. Prolonged and severe anemia and increased erythropoietic drive also result in hepatosplenomegaly (HSM) and extramedullary erythropoiesis.[13,14]

Clinical Presentations of Thalassemia Major:

Affected infants usually present at the age of 6 to 24 months by failure to thrive and become progressively pale with feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive HSM.[15]

In some developing countries, due to the lack of resources, patients are untreated or poorly transfused. The clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, genu valgum, HSM, leg ulcers and skeletal changes resulting from expansion of the BM. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth).[16,17]

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Laboratory Diagnosis:

Patients with thalassemia major have severe hypochromic microcytic anemia, associated with increased number of red blood cells (RBCs) and low mean corpuscular volume (MCV) and mean corpuscular Hb (MCH). Peripheral blood smears show hypochromia, anisocytosis, poikilocytosis, and nucleated red blood cells (i.e., erythroblasts) (figure 3, 4). The number of erythroblasts is related to the degree of anemia and is markedly increased after splenectomy.[14,18]

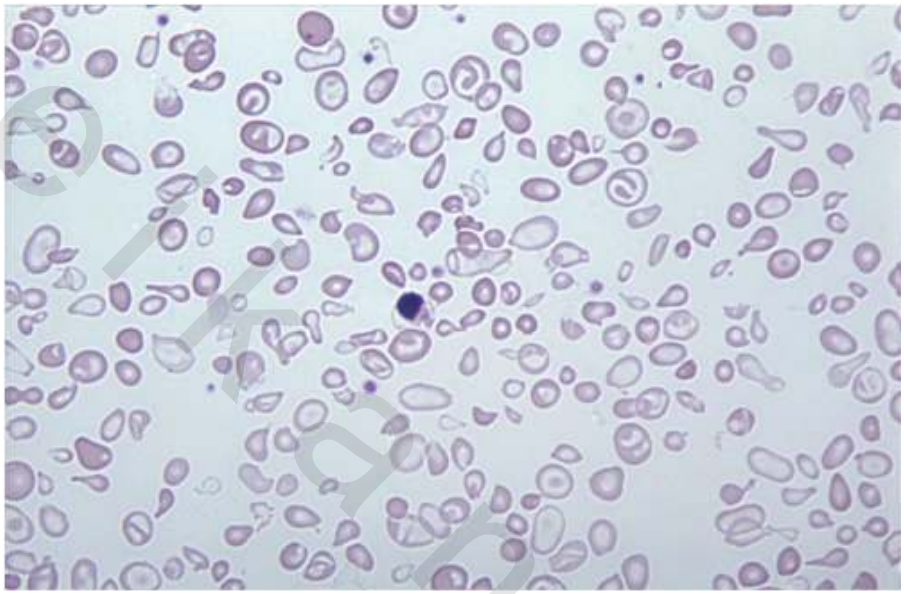

Figure 3: The peripheral blood findings in β thalassemia.[19]

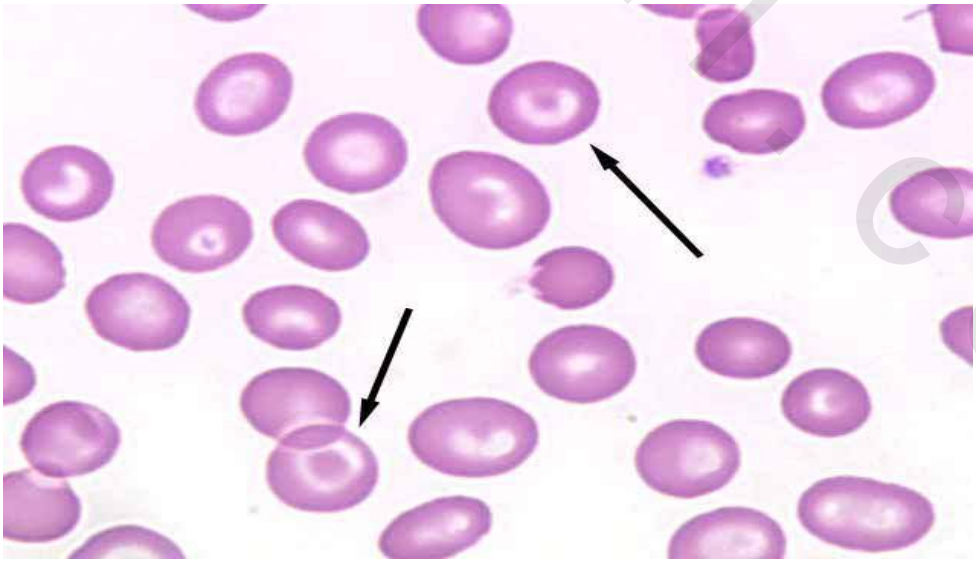

Figure 4: Target cells.[20]

Hemoglobin pattern by cellulose acetate electrophoresis or high-performance liquid chromatography (HPLC) varies according to the type of β thalassemia. In β^0 thalassemia, HbA is absent, HbF is 95–98%, and HbA_2 is 2–5%. In β^+ thalassemia, homozygotes with residual variable β globin synthesis, or β^0/β^+ compound heterozygotes, the Hb pattern shows HbA between 10 and 30%, HbF between 70–90%, and HbA_2 between 2–5 %.[21]

Transfusion Therapy of Thalassemia Major:

The goals of transfusion therapy are correction of anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption, which occurs in non transfused patients as a consequence of ineffective erythropoiesis.[22]

The decision to start transfusion in patients with confirmed diagnosis of thalassemia major should be based on the presence of severe anemia (Hb less than 8g/dl), excluding other contributory causes such as infections. However, in patients with Hb more than 8 g/dl, other factors should be considered, including facial changes, poor growth, and evidence of bony expansion and increasing splenomegaly. The decision to start regular transfusions should not be delayed after the third year due to the risk of developing multiple red cell antibodies and subsequent difficulty in finding suitable blood donors.[23]

Several different transfusional regimens have been proposed over the years, but the most widely accepted aims at maintaining a pre-transfusional Hb level between 9 to 10 g/dl and a post-transfusion level between 13 to 14 g/dl. This prevents growth impairment, organ damage and bone deformities, allowing normal activity and quality of life. The frequency of transfusion is usually every two to four weeks. The amount of blood to be transfused depends on several factors including weight of the patient, target increase in Hb level and hematocrit (Hct) of the blood unit.[24]

Types of Blood Products:

1- Packed Red blood Cells: (Figure 5)

Packed red blood cell units (PRBC) are manufactured by removal of the majority of plasma from a unit of whole blood. They have a volume of approximately 250 to 300 ml and a Hct of 65% to 80%. They are prepared without further modifications and contain white blood cells (WBCs), platelets, and residual plasma. One unit of PRBCs should raise the Hb of an average adult by 1 g/dL and the Hct by 3%. For pediatric patients, the usual dose given is 3 mL/kg to raise the Hb by 1 g/dL and the Hct by 3 %.[25]

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Figure 5: Packed RBCs.[26]

2- Washed Red Blood Cells:

They are a blood component obtained from whole blood after centrifugation, removal of the plasma and subsequent washing with isotonic solutions at + 4 °C. The Hct of this unit should remain between 65% and 75%. It can be prepared by either manual or automated methods. Automation is more efficient, resulting in loss of fewer RBCs with each wash cycle. When washing takes place in an open system, the product must be used within 24 hours.[27] The characteristics of washed RBCs are the removal of approximately 85% of the leucocytes, loss of about 15% of the RBCs, and loss of more than 99% of the donor original plasma which may be the target for antibodies in the recipient. [27]

Indications:

- The major indication for washed RBCs is the prevention of severe allergic transfusion reactions, thought to be mediated by recipient antibodies (most likely IgE) to donor plasma proteins.[28]
- Patients with IgA deficiency.[29]
- Prevention of allergic reactions not sensitive to antihistaminic drugs.[29]
- Post-transfusion febrile reactions, present even when leucodepleted RBCs are used.[30]

3- Leucodepleted Red Blood Cells:

Leukocyte-reduced RBCs are defined by the American Association of Blood Banking (AABB) as having less than 5×10^6 WBCs/unit. Early techniques of leucocyte reduction involved centrifugation, washing with saline, and removal of the buffy coat. Currently, filtration can be performed at the bed-side or in the laboratory with attachable filters that reduce leucocytes more than 99.9% with less than 10% depletion of RBCs.[31]

Consolidated Indications for Leucodepleted Blood Products :

1. Prevention of febrile non-haemolytic transfusion reactions (FNHTRs) caused by the presence of antibodies to WBCs.[32]
2. Reduction of the incidence of cytomegalovirus (CMV) infections in:[33]
    - CMV-negative patients with congenital or acquired immunodeficiency.
    - CMV-negative recipients of a bone marrow transplant (BMT) from a CMV-negative donor.
    - Pregnant women, independently of their CMV serological status, given the possible immunomodulatory effect of the transfusion (re-activation of CMV).
3. Reduction of the risk of rejection in candidates for hematopoietic stem cell transplantation (SCT).[33]
4. Prevention of refractoriness to platelet transfusion.[34]
5. Intrauterine transfusions and transfusions to premature babies, neonates, and pediatric patients up to 1 year old.[34]

Possible Indications

1. Candidates for renal transplantation: the use of leucodepleted RBCs prevents human leucocytic antigens' (HLA) alloimmunization and avoids the risk of transmission of CMV.[35]
2. In surgical patients, with the aim of preventing post-operative infections or recurrent neoplasm.[36]

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Timing of Leucofiltration

- **Laboratory (Pre-storage) filtration** refers to the filtration, at the blood bank laboratory, of packed red cells obtained by centrifugation of the whole blood (figure 6). It is the preferred method for leucoreduction. This method of leucocytes removal offers high efficiency filtration and provides consistently low residual leucocytes in the processed RBCs and high RBCs recovery.[37]

- **Bed-side (post-storage) filtration** refers to the PRBCs unit which is filtered at the bedside (figure 7), at the time of transfusion. This method, although equally sensitive to those above, may not allow optimal quality control such as measuring RBCs recovery and the residual leucocytic count.[37]

Figure 6: Laboratory pre-storage filtration.[38]

Figure 7: Bed-side filtration.[39]

Advantages of Pre-storage over Post-storage Leucoreduction:

- It eliminates the scope of inflammatory (interleukin-1, interleukin-6, tumor necrosis factor) cytokines accumulation during storage, and hence, is quite efficient in the prevention of FNHTRs.[40]

- It also minimizes the risk of HLA-alloimmunization in multitransfused patients, as it removes the intact leucocytes which are fragmented during storage and can pass through filters to alloimmunize the recipient against donor antigens.[41]

- Pre-storage leucofiltration can also minimize the risk of leucotropic virus transmission as leukocytes disintegrate and release the intracellular organisms after 72 hours of storage in blood components.[42]

- It is always easier to perform leucocyte quality control in the laboratory rather than the patient's bed-side. Hence during pre-storage leucoreduction, blood components can be thoroughly studied and evaluated for quality control such as Hb estimation of blood units and platelet count in the platelet units .[43]

4-Frozen (or Cryopreserved) Red Blood Cells:

It is the component derived from whole blood in which RBCs are frozen, preferably within 7 days of collection, using a cryopreservant and stored at -60^{o}C to -80^{o}C or below, based on the method used. These are used to maintain a supply of rare donor units for certain patients who have unusual red cell antibodies or who are missing common red cell antigens.(44)

Complications of Blood Transfusion:

1- Hemolytic Reactions:

A- Acute Hemolytic Transfusion Reactions (AHTRs):

These types of reactions usually occur due to incompatibility between donor RBC antigens and recipient plasma antibodies producing antigen–antibody complexes and causing complement fixation, intravascular haemolysis and ultimately destruction of the transfused blood. The severity of the reactions depends upon the recipient's antibody titer. Severe reactions are most often the result of ABO incompatibility and can be precipitated by transfused volumes of only a few milliliters.(45)

Symptoms manifest soon after starting the transfusion. In the conscious patient, they include headache, chest and flank pain, fever, chills, flushing, rigors, nausea and vomiting, urticaria, dyspnea and hypotension. In anaesthetized patients, these features may be masked and the first signs may be hypotension and features of increased blood hemolysis as haemoglobinuria and disseminated intravascular coagulation (DIC).(46)

B- Delayed Hemolytic Transfusion Reactions (DHTRs):

The donor RBC antigen–plasma antibody interactions responsible for this subset of transfusion reactions more commonly result from incompatibility with minor blood groups such as Kell, Kidd and Duffy. On pre-transfusion antibody screening, these patients commonly test negative because their antibody titre is too low to be detected. However, on further exposure to the antigen, a secondary immune response will be developed and after few days (7-21 days) the concentration of IgG antibody rises sufficiently to cause rapid destruction of those transfused RBCs that contain the foreign antigen.(47)

Antigen- antibody interactions of this nature do not activate the complement system, so extravascular rather than intravascular haemolysis occurs. The RBCs become coated with IgG and are then removed by the reticuloendothelial system (RES).(48)

Indicators of DHTRs are an unexpected reduction in Hct after transfusion, jaundice (unconjugated hyperbilirubinaemia) and a positive direct antiglobulin test (DAT).(48)

2- Allergic Reactions:

Allergic reactions are common and usually mild. They occur due to presence of foreign proteins in donor plasma and are IgE-mediated. Pruritus and urticaria, with or

without fever, are the most common features. The transfusion should be stopped and anti-histaminic drugs should be administered immediately.(46)

3- Febrile Non Hemolytic Transfusion Reactions:

The definition of FNHTRs includes a rise in temperature of at least 1°C (sometimes 1.5-2°C), preceeded by shivering and beginning 30–60 minutes after the start of the transfusion, which is not accounted for by the patient's clinical condition. Rarely they may progress to hypotension, vomiting and respiratory distress.(49)

Reactions result from donor leucocyte antigens reacting to antibodies present in the recipient's plasma. These antibodies react with the leucocytes to form a leucocyte antigen–antibody complex that binds complement and results in the release of endogenous pyrogens as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNFα).(50)

Some FNHTRs are not antibody-mediated, but component storage is an important factor in the occurrence of FNHTRs. Increased concentrations of cytokines, are harmful to the recipient, acting as endogenous pyrogens. Cytokines such as Interleukin-1 beta (IL-1ß), IL-6, Interleukin 8 (IL-8) and TNFα are actively synthesized and released during red cell storage. Linear correlations exist between cytokine level, white cell count and duration of storage. (51)

The effects of IL-1 include its potent pyrogenic activity, possibly mediated by IL-6 or prostaglandin E2 (PGE-2), stimulation of hemopoiesis, and activation of neutrophils and platelets. Tumour necrosis factor is also a potent pyrogen which enhances B cell proliferation. Interleukin- 6 is a pyrogen and also enhances antibody responses and stimulates B cell proliferation and differentiation. Interleukin -8 is a chemokine and a chemotactic factor for neutrophils and T cells. It stimulates neutrophil oxidative bursts and basophil histamine release. These findings support the concept that proinflammatory cytokines play a role in FNHTRs.(51)

4- Transfusion Related Infections:

A- Bacterial:

Bacterial contamination of blood components is an infrequent complication of transfusion. If it does occur, the potential for fulminant sepsis in the recipient is associated with high mortality. It can result from contamination during venipuncture or if an asymptomatic donor is bacteraemic at the time of donation. Symptoms occur during or shortly after transfusion of the contaminated unit and include high fever, rigors, erythema and cardiovascular collapse.(52)

Red blood cells are stored at 4°C. This makes contamination with Gram-negative bacteria such as *Yersinia enterocolitica* and *Pseudomonas* species more likely as they proliferate rapidly at this temperature. Gram-positive bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* species proliferate more readily at room temperature and so are less commonly seen.(52)

B- Viral:

The incidence of transfusion-related viral infection has been greatly reduced when pre-donation questionnaires to identify groups with high-risk behaviour were implemented. There have also been improvements in pre-transfusion testing of donated blood. Currently, in Egypt, blood donors are screened for hepatitis B (HBV), hepatitis C (HCV), human immunodeficiency virus (HIV) 1, 2 and syphilis. However, disease transmission may occur in the 'window period',[45]

C- Parasitic [53]

Several parasites can be transmitted by blood transfusion as:

- Plasmodium species (malaria).
- Trypanosoma cruzi (Chagas' disease).
- Toxoplasma gondii: only a risk in immunosuppressed patients transfused with granulocytes.
- Babesia microti (Nantucket fever).
- Leishmania donovanii.

5- Transfusion-Related Acute Lung Injury (TRALI):

It is the most common cause of major morbidity and death after transfusion. It presents as an acute respiratory distress syndrome (ARDS) either during or within 6 hours of transfusion in the form of hypoxemia, dyspnoea, cyanosis, fever, tachycardia and hypotension which result from non-cardiogenic pulmonary oedema. Immune TRALI results from the presence of leucocyte antibodies in the plasma of donor blood directed against HLA and human neutrophile alloantigens (HNA) in the recipient. Non-immune TRALI occurs due to reactive lipid products released from the membranes of the donor blood cells. [54]

Human Leucocytic Antigens:

Human leukocyte antigens (HLA) are the human version of the major histocompatibility complex (MHC). They consist of a family of cell surface polymorphic molecules involved in the presentation of antigens to T cells and therefore, play a central role in the induction and regulation of immune responses.[55]

- **Genetics:**

The genes coding for the HLA molecules are located on the short arm of chromosome 6 and span a distance of approximately 4 megabases (Mb).[56]

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- **Classification According to Structure:**

Class I: (Figure 8)

The HLA class I genes have been classified according to their structure, expression and function as classical (HLA-A, -B and -C) and non-classical (HLA-E, -F and -G). Both classical and non-classical HLA class I genes code for a heavy α chain, of approximately 43 kilodalton (kDa), non-covalently linked to a non-polymorphic light chain (β2-microglobulin) of 12 kDa, which is encoded for by a gene on chromosome 15.[57]

The extracellular portion of the heavy chain has three domains (α 1, α 2 and α 3) approximately 90 amino acids long. The α 1 and α 2 domains are the most polymorphic domains of the molecule and they form a peptide-binding groove that can accommodate antigenic peptides approximately eight to nine amino acids long.[57]

Class II: (Figure 8)

The class II DPA and DPB, DQ and DR genes code for a heterodimer formed by two noncovalently associated α and β chains of approximately 34 and 28 kDa respectively. The expressed α and β chains consist of two extracellular domains as well as transmembrane and cytoplasmic domains.[58]

The majority of the polymorphism is located in the β1 domain of the DR molecules and in the α1 and β1 domains of the DP and DQ molecules. Similarly to class I molecules, these domains also form a peptide-binding groove. However, in case of the class II molecules (DR), the groove is open at both sides and it can accommodate antigenic peptides of varying size, although most of them are approximately 13–25 amino acids long.[58]

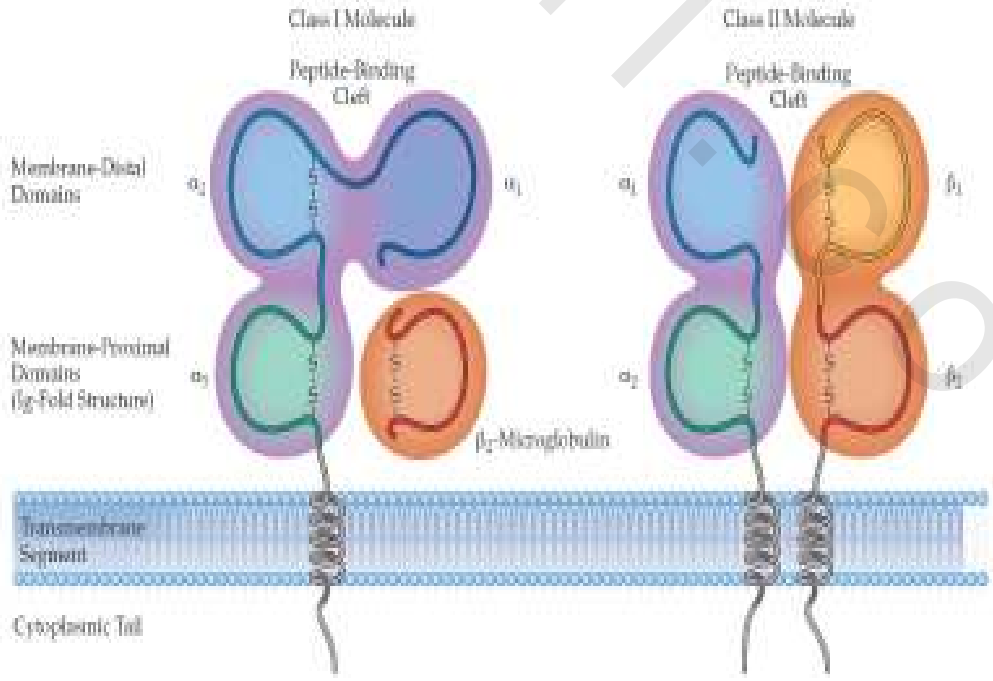

Figure 8: The two structural types of MHC molecules; class I and II.[59]

Class III

Class III subregion lies between the other two subregions and contains genes coding for a diverse group of proteins, including complement components (C4Bf), TNF and heat-shock proteins.[60]

- **Distribution:**

The classical HLA class I molecules (A, B, C) are expressed on the majority of tissues and cells, including T and B lymphocytes, granulocytes and platelets. Low levels of expression have been detected in endocrine tissue, skeletal muscle and cells of the central nervous system (CNS) .[61]

Class II molecules are expressed on B lymphocytes, antigen-presenting cells (APCs); (monocytes, macrophages, and dendritic cells) and activated T lymphocytes.[61]

- **Function:**

HLA molecules provide the crucial surface upon which the antigen receptors on T lymphocytes recognize foreign (non-self) antigens (figure 9). On APCs, class II molecules present antigenic fragments to the CD4+ inducer (or helper) T cells, while class I molecules function at the effector phase of immunity by presenting antigens to CD8+ T cells, which generally have cytotoxic or suppressor function. This process of antigen presentation consists of the binding of a single T cell receptor (TCR) to a complex on the surface of APCs consisting of the MHC molecule and a peptide fragment derived from the foreign antigen.[62]

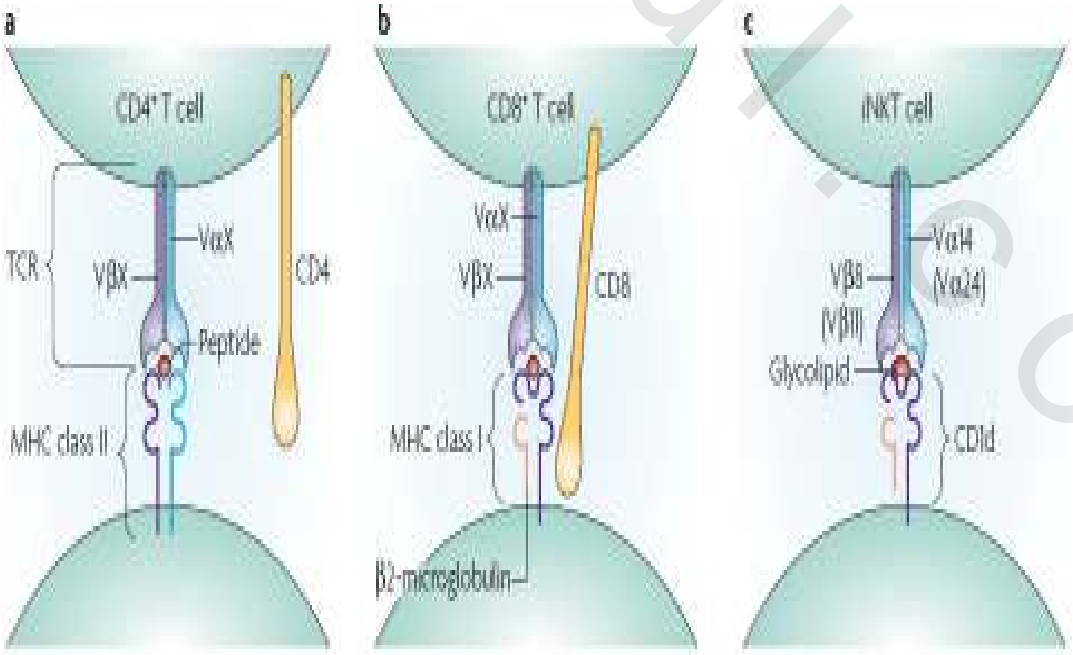

Figure 9: Antigen recognition by T cells. The "X" represents variable T cell receptor chains.[63]

- **Mechanism of HLA Alloimmunization: (Figure 10)**

Because of their high density on the surface of cells, HLA molecules can become immunogenic when cells from one individual are exposed to another's immune system. The mechanisms leading to HLA allosensitization are believed to follow two pathways. The first pathway is followed during most immune reactions and involves donor antigen uptake by recipient APCs and presentation to recipient lymphocytes (indirect pathway). In this case, the donor's HLA molecules are processed into peptides through the exogenous pathway of antigen presentation and presented to recipient T cells as linear peptides. This pathway is believed to be responsible for the development of alloantibodies.[64,65]

Because the function of HLA molecules is to present antigenic determinants to T cells, it could be easily envisioned how minor changes in their structure could be misinterpreted as antigenic epitopes. Thus, intact HLA molecules residing on the surface of donor cells are a perfect target for T cell–mediated allorecognetion (direct pathway) by recipient cells either through direct cytotoxic effect of T cells against target cells or by the activation of helper T cells, through HLA class II engagement, which leads to stimulation of antibody- mediated immune responses.[64]

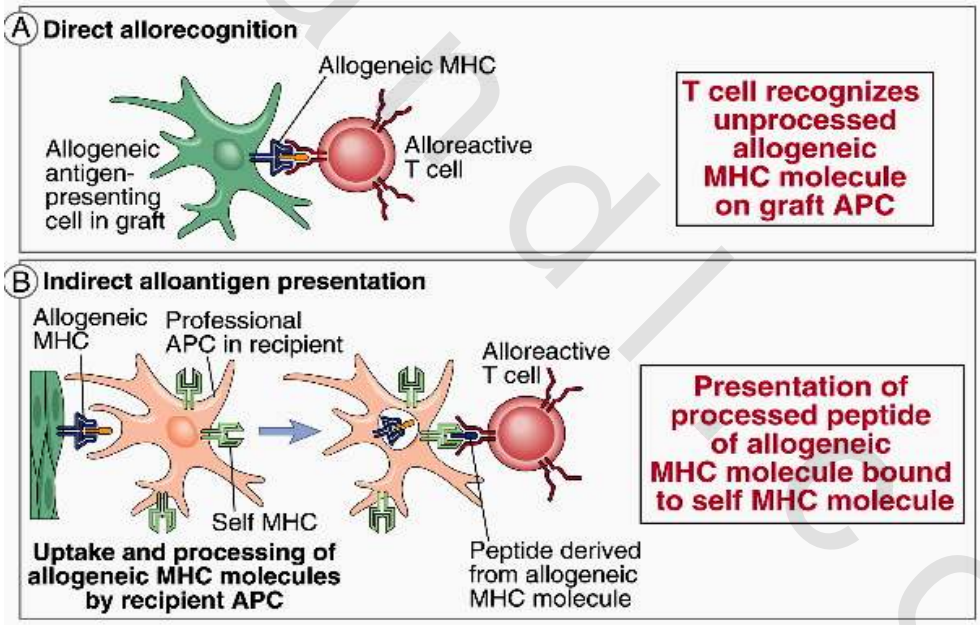

Figure 10: Direct and indirect pathways of allorecognetion.[66]

HLA Antibodies:

HLA specific antibodies may be produced in any situation that exposes the host to these alloantigens including pregnancy, transplantation, blood transfusion and planned immunization. Cytotoxic HLA antibodies can be identified in approximately 20% of human pregnancies. The antibodies produced are normally multispecific, high titer, high affinity and of the IgG class. The majority of HLA antibodies found in multitransfused patients are multispecific IgM and IgG.[67]

- **Methods for HLA Antibody Detection**:

1-Complement-dependent Lymphocytotoxicity Test

The complement-dependent cytotoxicity (CDC) test involves mixing equal volumes of serum and cells to allow the binding of the specific antibody to the target cell followed by the addition of rabbit complement. Complement-fixing antibodies reacting with the HLA antigens present on the cell surface lead to the activation of complement via the classical pathway and result in the disruption of the cell membrane. The lysed cells are then detected by adding ethidium bromide (EB) and the live cells are identified by adding acridine orange (AO) at the end of the incubation period. Live cells stained with AO when exposed to ultraviolet (UV) light, appear green, whereas lysed cells allow the entry of EB which binds to DNA, and they appear red under UV light (figure 11). The results are expressed as the percentage of the panel cells that are reactive; this is called the percent panel reactive antibody (%PRA).[68]

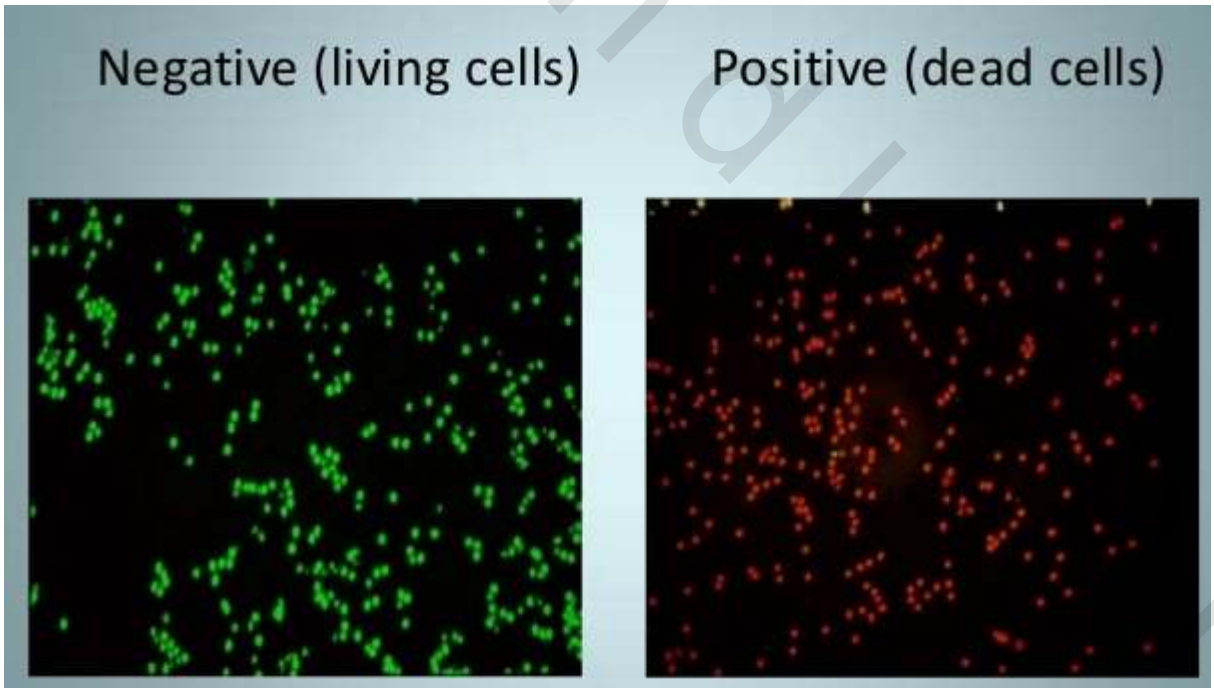

Figure 11: Positive and negative cells in CDC test.[69]

2- Luminex HLA Antibody Screening Technology:

It consists of a series of polystyrene microspheres (beads), containing fluorochromes of differing intensity which give each group of beads attached to HLA molecule or molecules a unique signal.

There are three levels of bead attachment. The first level consists of beads bound with a large number of class I or class II molecules which essentially provide a positive or negative result. At the second level, the bead is equivalent to a cell, with each bead containing two molecules derived from two alleles at each locus; HLA-A, -B and -C in the case of class I and HLA-DR, -DQ in the case of class II. The third level consists of beads with one HLA molecule attached (either class I or II) referred to as a single antigen bead (SAB). This level is particularly useful for characterizing complex sera with a high panel reactive antibody (PRA), accurately defining the antibodies present.

Test sera are added to the bead mix and the HLA antibodies bind to the bead with the appropriate HLA molecule attached. A second phycoerythrin (PE) -labelled anti-human IgG antibody is then added which binds to the primary HLA antibody. When the sample is passed through the detector, one laser excites the fluorochrome in the bead which exhibits a unique signal and the other laser excites PE bound to the second antibody. The combination of these signals defines the specificity of the antibody in the test serum.[70]

3- Enzyme Linked Immunosorbent Assay (ELISA)

ELISA-based methods have often been the technique of choice for antibody detection for a number of antigen systems, particularly where there has been a requirement for testing large numbers of samples.

The basic principle of the technique is as follows: HLA antigens are purified and immobilized on a microwell plate, directly or via an antibody directed against a non-polymorphic region of the HLA antigen. HLA-specific antibodies bound to the immobilized antigen can be detected with an enzyme-linked secondary antibody which, upon addition of specific substrate, catalyzes a colour change reaction that is detected in an ELISA reader.

One of the main advantages of ELISA technique is that the non-cytotoxic antibodies detected are HLA specific since it relies on the binding of the antibodies to wells coated with pools of solubilized HLA antigens.[71]

4- Flow Cytometry

In this technique, cells and serum are incubated to allow the binding of the antibody to the target antigen. The bound antibody is then detected by using an antibody against human immunoglobulin labelled with a fluorescent marker such as fluorescein isothiocyanate (FITC) or R-PE. At the end of the incubation period, the cells are passed through the laser beam of the flow cytometer to identify the different cell populations based on their morphology/granularity and on the fluorescence.

The main advantages of flow cytometric techniques are the increased sensitivity when compared with LCT- and ELISA-based techniques and the detection of non-complement-fixing antibodies, allowing early detection of sensitization. However, one of the disadvantages is that it also detects non-HLA lymphocyte-reactive antibodies which are of unclear clinical relevance.[72]

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