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Alloimmunization among Transfusion-Dependent Thalassemia Patients in the West Bank

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Thesis Approval

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Dedication

This thesis is dedicated to the following people:

My great parents, Aysha and Abdelrahman, who never stopped giving of themselves in countless ways.

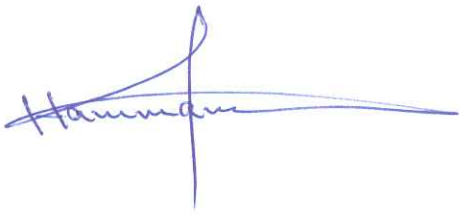
My dearest wife, Sanaa, who led me through the valley of darkness with the light of hope and support.

My beloved brothers and sister; particularly my great brother Husni, who stood by me when things looked bleak.

All of the thalassemia patients in Palestine, who needed this re

Declaration:

I Certify That This Thesis Submitted for The Degree of Master, is The Result of My Own Research, Except Where Otherwise acknowledged, and That This Study (or Any Part of The Same) Has Not Been Submitted for A Higher Degree to Any Other University or Institute.

Signed: 

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Abstract

Introduction:

Red blood cell transfusion has greatly reduced the mortality and morbidity in multiply transfused thalassemia patients. However, this can result in red blood cell isoimmunization with alloantibodies which can lead to serious complications such as acute and delayed hemolytic transfusion reactions.

Objectives:

To assess the frequency and types of alloantibodies in transfusion-dependent thalassemia patients in the middle and southern regions of the West Bank. Furthermore, to assess the association between alloantibody development and gender, age of first transfusion, blood type, splenectomy, frequency of transfusion.

Methods:

This cross-sectional study was performed between February and June, 2017 at three Thalassemia Centers in the southern and middle districts of the West Bank. A total of 101 transfusion-dependent thalassemia patients from these centers were included. Clinical and transfusion records were examined for age of patients, age at first transfusion therapy, total number of blood units transfused, and the status of spleen. Alloantibody screening and identification was also performed by using the gel card method (Diamed ID, Switzerland).

Results:

Eleven out of one hundred and one participants (10.9%) had alloantibodies. Ten of them (90.9%) were diagnosed with β-thalassemia major, and one (9.1%) was diagnosed with β-thalassemia intermedia. The majority of alloimmunized patients were females (8; 72.7%).

Eight patients (72.7%) were splenectomized. Seven patients (63.6%) had single alloantibody, while four (36.4%) developed multiple alloantibodies.

Six types of alloantibodies were identified, three (50%) were against antigens from the Rh system (anti-E, anti-C and anti-D), two (33.3%) were against antigens from the Kell system (anti-K and anti-Kp^a) and one (16.7%) was against an antigen from the Kidd-system (anti-Jk^a).

Conclusions:

This data showed quite high alloimmunization rate among transfusion-dependent thalassemia patients in the middle and southern regions of the West Bank. The most frequently detected alloantibodies were against the Rh and Kell antigens.

In order to reduce the alloimmunization in these patients, a policy to perform extended red cell phenotyping of the patients and issuing antigen-matched blood should be adopted.

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List of Abbreviations

AHG: Anti-Human Globulin

ATP: Adenosine Tri-Phosphate

ATR: Acute Transfusion Reaction.

CPD: Citrate Phosphate Dextrose

CPDA: Citrate Phosphate Dextrose Adenine.

DAT: Direct Anti-globulin Test

DHTR: Delayed Hemolytic Transfusion Reaction

DTR: Delayed Transfusion Reaction.

EDTA: Ethylene Diamine Tetraacidic Acid

ESR: Erythrocyte Sedimentation Rate .

FDA: Food and Drugs Administration

GVHR: Graft Versus Host Reaction

HLA: Human leukocyte antigen

IAT: Indirect Anti-globulin Test

IgG: Immunoglobulin G

ISBT: International Society of Blood Transfusion

LISS: Low Ionic Strength Saline.

MHC: major histocompatibility complex

miRNA: micro-Ribo-Nucleic Acid

NNUH: an-Najah National University Hospital

PEG: Polyethylene-Glycol

PMOH: Palestinian Ministry of Health

RBC: Red Blood Cell

SHOT: Serious Hazards of Transfusion

SNPs: Single Nucleotide Polymorphisms

TIF: Thalassemia International Federation

TPFS: Thalassemia Patients Friends Palestinian Society.

WBC: White Blood Cell

Chapter One

Introduction

Complications due to erythrocyte alloimmunization make transfusion therapy problematic in transfusion-dependent patients, including thalassemia patients.

The Food and Drugs Administration (FDA) identifies non-ABO antibodies as the second leading cause of mortality associated with blood transfusion in the period from 2005 to 2013 in the United State of America (USA) (FDA, 2012). Serious Hazards of Transfusion "SHOT" indicates that alloimmunization and hemolytic transfusion reactions are the second most common pathologic complication from transfusion in the United Kingdom (UK) (SHOT, 2015).

In developing countries, regular blood transfusion remains the main treatment for thalassemia, so the mortality associated with transfusion due to alloimmunization in these countries could be higher than that in the UK or USA, though there are no data available about mortality associated with transfusion due to alloimmunization. In these countries, complete phenotype screening for recipient patients and transfused red blood cell units are not routinely done. Only ABO and Rh-D typing is routinely done before transfusion with the indirect anti-globulin test -IAT. The lack of knowledge of other blood group system screening methods could lead to an increase in the frequency of alloimmunization in such

countries. However, no data are available about the frequency of erythrocyte alloimmunization in transfusion-dependent thalassemia patients in the State of Palestine.

Following transfusion, pregnancies/delivery or transplantations, exposure to foreign RBC antigens may occur, which could lead to an immune response and antibody production against foreign RBC antigens. This immune response is called alloimmunization while the related antibodies are called alloantibodies.

In blood transfusion, the first exposure to foreign antigens stimulates the immune system which produces corresponding antibodies against this foreign antigen. This first immune response does not lead to hematological complications, but with time, the concentration of these alloantibodies is decreased and will be undetected by immuno-hematological tests. The second exposure to the same antigen results in a secondary immune response and leads to the production of high amounts of gamma immunoglobulins (IgG) which sensitize the transfused red cells. This sensitization leads to a delayed hemolytic transfusion reaction. This hemolysis leads to an increase in the medical complication of anemia for the recipient. Moreover, the presence of alloantibodies makes it difficult to provide compatible red blood cell units for transfusion.

Thalassemia is one of the most common hemoglobinopathies worldwide. About 60,000 children are annually born affected by β-thalassemia (TIF, 2007). The first description of thalassemia was done in 1925 in Detroit by Thomas Cooley.

Thalassemia is a diverse group of congenital hematological diseases which occurs as a result of mutation or mutations in one or more globin genes. These mutations lead to the decrease or absence of the synthesis of corresponding globin chains. The most common types are α and β thalassemia which are considered the result of mutations in α or β genes which affect hemoglobin A (the major adult form of hemoglobin).

In normal hemoglobin A, the α chain to β chain ratio is one to one but in β thalassemia, the mutations in the β gene lead to a reduction or even absence in the amount of β globin chain which leads to an excess in the amount of α chain. Excess α chains bind to erythrocyte membranes which causes a decrease in erythrocyte deformability and erythrocyte membrane damage. These excess α chains are the major source of anemia in β thalassemia. In the bone marrow, macrophages destroy precipitate-filled erythrocytes which lead to ineffective erythropoiesis. Moreover, circulating erythrocytes with excess precipitate α chains are removed by the spleen in the extravascular hemolysis mechanism (Hoffbrand, Higgs, Keeling, & Mehta, 2016; McKenzie, Williams, & Landis-Piwowar, 2015).

Beta thalassemia is classified depending on its severity. This classification includes: major thalassemia, which is defined as a type of thalassemia with severe clinical phenotype due to homozygote mutation in the beta-globin gene; intermedia thalassemia, which is the type of thalassemia with intermediate clinical phenotype because the beta gene still can encode some beta chains; and minor thalassemia, which is the type of thalassemia that is also called thalassemia trait because one normal beta globin gene is present and, as a result, mild clinical phenotype is presented (Jha R, 2014).

Patients with major β thalassemia need medical attention and require regular blood transfusion within the first two years of life. The goal of regular blood transfusion is to maintain the hemoglobin level between 9.5 and 10.5 g/dl which allows for normal developmental growth (Jha R, 2014). Lifelong blood transfusions are needed for major β thalassemia patients which leads to an increase in the side effects of blood transfusions such as iron overload, transfusion-transmitted infections and delayed hemolytic transfusion reactions due to the presence of alloantibodies. This study focuses on alloimmunization in transfusion-dependent thalassemia patients in the West Bank.

Depending on recently updated data from Thalassemia Patients Friends Palestinian Society (TPFS), in the West Bank, there are 566 thalassemia patients; 500 (88%) of them are transfusion-dependent while the other 66 cases were treated by bone marrow transplantation outside the State of Palestine. Red blood cell transfusion remains the main treatment available for thalassemia patients in the State of Palestine (TPFS, 2017).

1.1.Problem Statement

Alloantibodies against non-ABO antigens are identified as the second cause of mortality associated with blood transfusion by FAD and SHOT in the USA and the UK respectively (FDA, 2012; SHOT, 2015).

These antibodies are formed after exposure to foreign erythrocyte antigens which stimulate the immune system to produce antibodies against these antigens. These alloantibodies can be detected by pre-transfusion testing. The reaction due to the presence of alloantibodies could occur in 2.6% of transfusions.

Alloantibody formation is common in chronic transfusion-dependent patients as well as in hematological malignancies and hematological diseases such as thalassemia and sickle cell diseases. In the West Bank, thalassemia is the most common type of hemoglobinopathy.

Recommendations for compatibility screening of phenotypes for blood group systems other than ABO and Rh-D could reduce the frequency of alloimmunization for these patients and avoid any complications. This would also allow the patients to get the utmost benefits from transfused units.

1.2. Study Justification

This research study aims to screen the blood of thalassemia transfusion–dependent patients for circulating antibodies in order to identify them. The study also aims to develop recommendations for health authorities to start screening blood units for the presence of these particular antigens in order to give patients fully matched blood which helps them avoid any complications. This will allow them to get the utmost benefit of the transfused units.

1.3. Objective

1.3.1. Main objective:

The main objective of our research is to determine the frequency and identification of alloantibodies among transfusion-dependent thalassemia patients in the West Bank.

1.3.2. Specific objectives:

The first specific objective of our research is to identify the frequency of each alloantibody in transfusion-dependent thalassemia patients in the West Bank.

The second specific objective of our research is to assess the association between development alloantibody and factors such as gender, age at the first transfusion, ABO and Rh blood group, splenectomy, and frequency of transfusion.

1.4. Hypotheses

Tow hypotheses were consider. First, that there is no alloimmunization among transfusion-dependent thalassemia patients in the West Bank. Second, there is no significant association between alloimmunization and Splenectomy, gender, frequency of transfusion, number of transfused units, blood group and age.

Chapter Two

2. Literatures Review

2.1.Introduction

From the beginning of history, humans have been interested in blood: the ancient Egyptians bathed in blood, blood was drunk by aristocrats, and authors and artists have used blood as themes. Nowadays, in modern medicine, blood transfusions are a cornerstone for the treatment of many diseases and medical cases and transfusion medicine is rapidly developing (Harmening, 2012). However, the risks and side effects of blood transfusion have also developed beside the wide use of blood and blood products in medicine, and therefore should be minimized. One of these risks is the presence of alloantibodies against foreign red cell antigens and the delayed hemolytic transfusion reaction which is associated with these types of antibodies. Alloantibodies minimize the availability of compatible units for patients, which could lead to complicating the patient's condition.

2.2.History of Transfusion

The first blood transfusion process in history was done in 1492 when blood was transferred from three volunteer donors to Pope Innocent VII, who was bleeding. The four people involved died in the blood transfusion process. In 1818, James Blundell and Thomas published the first report of a blood transfusion by syringe. Clotting formation hold-back

lead to failing these historical blood transfusion processes, but this was solved by Braxton Hicks, who recommended in 1869 using sodium phosphate as a non-toxic anticoagulant. In 1914, sodium citrate was reported by Hustin as an anticoagulant for transfusion, and in 1916, citrate dextrose solution was identified in blood preservation by Rous and Turner. In 1943, acidic citrate dextrose (ACD) as a blood preservative solution was identified by Loutit and Mollison. In 1957, Gibson identified citrate phosphate dextrose (CPD) for blood preservation. Citrate phosphate dextrose consists of citrate and dextrose; citrate plays a role in calcium chelation to prevent clot formation and dextrose plays a role in cellular energy by providing adenosine triphosphate ATP. In 1979, Ernest Beutler and Carol West suggested that the addition of adenine and increasing the amount of glucose to CPD provides more stability for blood units by persevering which is called citrate phosphate adenine (CPDA). The main goal of blood preservation is to provide functional and viable blood components for patients, and according to FDA identified criteria, free hemoglobin should be less than 1% and the average 24-hour post-transfusion erythrocyte survival should be more than 75%. Under these criteria, blood unit stability depend on the type of preservative, as CPD is stable for 21 days whereas CPDA-1 is stable for 35 days at 1-6^{o}C (Beutler & West, 1979; Blundell, 1818; Harmening, 2012).

In 1869, Adolf Criete reported that after mixing red blood cells from one species with serum from another species, the red blood cells form an agglutination (Harmening, 2012). The difference in red blood cell agglutination was described by Austrian pathologist Karl Landsteiner in 1901 and led to the ABO blood group system and its serious reactions that could occur as a result of an incompatible transfusion. In 1911, Reuben Ottenberg introduced the ABO blood grouping test to select compatible donors, which led to a reduction in fatalities from blood transfusion in the early days after transfusion and recommended to perform cross-matching beside ABO blood grouping as routine pre-

transfusion testing. However, the Ottenberg cross-matching technique needed 10-15 ml of blood and 2 hours incubation, so later on, Ross and Lee improved the cross-matching technique by reducing the blood volume and incubation time to less than 15 minutes (Lee, 1917; Rous & Turner, 1915). The first non-ABO antibody was identified in 1921 by Under (Unger, 1921). These irregular antibodies were detected well after 30 minutes of incubation at 37^{o}C, this suggestion was done by Levine and Diamond who suggested that bovine albumin enhances aggregation. In 1940, an antibody was reported by Landsteiner and Wiener from pigs and rabbits when they were transfused with Rhesus macaque monkey RBCs. This antibody was agglutinated 85% of human RBCs and was termed Rh for Rhesus monkey (Harmening, 2012).

In 1945, Coombs introduced a new test to detect weak and incomplete agglutinins called the indirect antiglobulin test. The indirect antiglobulin test added a new dimension to the safety of blood transfusion and lead to a rapid increase in the identification of alloantibodies that caused transfusion reactions (R. R. Coombs, Mourant, & Race, 1945).

To further increase transfusion safety, sensitive cross-matching protocols were developed, including direct antiglobulin tests and auto-controls. Enzyme-treated red blood cells and additives such as bovine albumin, low ionic strength media and polyethylene-glycol (PEG) were used to enhance agglutination and to further shorten incubation times. Using these sensitive techniques, many new blood group systems were discovered. This revolution in blood transfusion and transfusion medicine in the 20^{th} century led to massive blood use which caused circulatory overload in many patients. This problem was solved by blood component therapy, where each blood component is available as a concentrated form such as packed red blood cells, concentrated platelets and cryoprecipitate (rich in anti-hemophilic factors).

2.3.Blood Group Systems

In human erythrocytes, 322 different antigens in 36 different systems have been identified by The International Society of Blood Transfusion (ISBT, 2016). These systems include the ABO system which includes A antigen and B antigen, the Rh system which includes D, C, c, E and e antigens, … etc. (Appendices 1 and 2).

The most recently discovered system is the Augustine system, which includes four antigens. The Rh system is the biggest blood group system which includes 55 antigens, followed by the MNS system with 49 antigens (ISBT, 2018).

Erythrocyte antigens have different structures that lead to a diversity in their functions. These antigens reside on erythroid membrane glycoproteins or glycolipids, though they are not erythroid-specific antigens and could be present on the surface of other cells.

Erythrocyte antigens have a biological function; a transporter function with Colton antigens (CO) playing a role as a water channel, Kidd antigens (JK) playing a role as a urea transporter, and Diego antigens (DI) playing a role as an anion exchanger. Erythrocyte antigens function as receptors, such as Duffy antigens (FY) and Knobs antigens (KN), and have adhesion functions such as Indian antigens (IN) and Landsteiner-Wiener antigens (LW). Finally, erythrocyte antigens have enzymatic functions such as Kell antigens (KEL) and YT antigens Zn-Metalloproteinase and Acetylcholinesterase respectively. Other antigen systems such as the Scianna system and Raph system have unknown functions. (Cartron & Colin, 2001)

Genetically, blood group antigens are encoded by the same gene classified in the same blood group system where each blood group system could consist of one or more antigens. Most blood group polymorphisms are the result of single nucleotide polymorphisms

(SNPs) encoding amino acid substitutions in an extracellular domain of an RBC surface protein (Storry & Olsson, 2004).

2.4.Pretransfusion Testing

The ABO and Rh blood grouping test is the key test for blood transfusion and major cross-matching by mixing donor RBC suspensions with the patient's serum to ensure there are no antibodies present in their plasma against the RBC antigens of the donor.

However, the major cross match test cannot detect alloantibodies in all titrations because alloantibodies after a certain period of time are undetectable by in vitro testing. Phenotyping for patients and donors could minimize the formation of these alloantibodies. The formation of alloantibodies is variable between individuals, so the human population is classified as non-responder, responder and hyper responder depending on the susceptibility to alloantibody formation after exposure to foreign antigens (Gehrie & Tormey, 2014).

2.4.1. Antiglobulin Test:

The antiglobulin test, also known as the Coombs test, depends on the antihuman globulin AHG obtained from immunized non-human species binding to human globulins such as IgG and complements in free form in serum or binding form to erythrocyte antigens. The first description of this test was done by Coombs to detect weak or non-agglutinating Rh-antibodies in 1945 (R. R. Coombs et al., 1945).

The anti-human globulin test detects incomplete or non-agglutinating antibodies such as IgG_1 and IgG_3 because they are monomers that are too small and cannot directly agglutinate sensitized RBCs. Therefore, adding antihuman globulin to RBCs, which are

sensitized by incomplete antibodies, leads to the hemagglutination of these sensitized RBCs, helping to observe the undetectable formation of the antibody-antigen complex.

In 1946, Coombs, and co-workers suggested that the antihuman globulin test could be used to detect the in vivo sensitization of the RBCs of newborns which leads to hemolytic disease (R. Coombs, Mourant, & Race, 1946). The anti-human globulin test is classified into two tests, the direct and indirect antihuman globulin tests. This classification depends on the sensitization environment, with the direct antihuman globulin test detecting sensitized RBCs in vivo while the indirect antihuman globulin test detects sensitized RBCs in vitro (Harmening, 2012).

Various technical modifications have increased the sensitivity of the detection of antibody-coated RBCs, such as using more specific AHG as mono-specific AHG against IgG or complement C3. Moreover, one of these modification techniques is enzyme elution of the antibodies from the coated RBCs (Alwar, Devi, Sitalakshmi, & Karuna, 2012).

In 1990, the first description of gel techniques using antibody coated RBCs was done by Lappierre (Lapierre et al., 1990). This new technique is based on the Sephadex gel matrix which acts as a sieve, where agglutinated RBCs are too large to pass through and are trapped in the gel matrix. The negative reaction is observed as a clear pellet of cells that have settled at the bottom of the microtube. But this new technique still has a limitation with 60.46% specificity and 83.01% sensitivity. False positive results occur due to high Erythrocyte Sedimentation Rate ESR, macrocytosis, and marked leukocytosis, so positivity by the Sephadex gel matrix method needs to be correlated with the clinical presentation of the patient and other laboratory findings and a positive result needs to be confirmed by monospecific antisera (Alwar et al., 2012).

2.4.2. Crossmatch:

One of the application tests of the indirect Coombs test is a crossmatch test. This test detects the patient's antibodies sensitizing donor erythrocyte antigens in vitro to suspect this process in vivo. A negative or compatible crossmatch test (no sensitized donor RBCs by recipient antibodies) indicates safe blood transfusion for the patient.

2.4.3. Antibody Identification:

Another application test of the indirect Coombs test is an antibody identification test. This test is done after detection antibody or antibodies in the patient's serum. By using a commercial panel of cells with known antigens, the antibody or antibodies present in the patient's serum can be identified. About 30-40% of alloantibodies are undetectable months or even years after their first identification, (Calabro et al., 2016) and there are many techniques available to enhance agglutination reactions such as the low ionic strength saline (LISS) technique, the albumin and papain technique and the polyethylene glycol (PEG) technique. The polyethylene glycol antiglobulin technique was reported as the most sensitive technique (Pineda, Vamvakas, Gorden, Winters, & Moore, 1999).

2.5. Risks associated with Transfusion

Blood transfusion is a life-saving procedure, though it involves risks. Some of these risks could be prevented by testing, while others cannot but can be managed under medical observation. Transfusion reactions are classified by the interval time between the transfusion and reaction occurring in two groups: acute transfusion reactions (ATR) and delayed transfusion reactions (DTR).

2.5.1. Acute Transfusion Reactions:

Acute transfusion reactions are defined as any transfusion reaction occurring with signs and symptoms present within 24 hours post-transfusion.

One of the acute transfusion reactions is the acute hemolytic transfusion reaction which is a hemolytic complication post-transfusion within 24 hours. These reactions are classified into immune and non-immune origin. For immune origin, a small amount of incompatible blood can cause rapid hemolysis. The severity of symptoms is related to the amount and rate of incompatible blood transfused in which the most severe reaction is associated with ABO incompatibility. ABO incompatible transfusions are estimated to occur in one in every 38,000 - 70,000 RBC transfusions.

Acute hemolytic transfusion reaction symptoms include back and flank pain, hemoglobinuria, hemoglobinemia, hypotension, renal failure and diffuse intravascular coagulopathy. Non-immune origin acute hemolytic transfusion reactions include transfusion associated-sepsis. Moreover, the febrile non-hemolytic transfusion reaction is an acute transfusion reaction with a high frequency of 0.12-0.5% of transfused RBC units (Harmening, 2012; Kaushansky, 2016).

2.5.2. Delayed Transfusion Reactions:

Any transfusion reaction occurring post-transfusion with signs and symptoms that appear after 24 hours is defined as a delayed transfusion reaction. One delayed transfusion reaction is the delayed hemolytic transfusion reaction (DHTR). This type of transfusion reaction occurs as a result of the presence of alloantibodies, but in undetectable concentrations, so the cross-match result appears compatible. This occurs as a result of a special characteristic of alloantibodies, as 30-40% are undetectable after months to years of their first identification. This type of transfusion reaction occurs in 0.2-2.6% of transfused

patients and is rare in infants younger than 4 months but common in chronically transfused patients. A first transfusion or pregnancy can lead to immunization of the patient, while a second transfusion or pregnancy with re-exposure to a blood group antigen can lead to a secondary immune response with the rapid production of IgG antibodies. These antibodies sensitize RBCs and lead to extravascular hemolysis, which appears as a decrease in hematocrit and hemoglobin levels, which could be noted days to weeks after transfusion. This is also associated with unexplained fever. The delayed hemolytic transfusion reaction is mostly extravascular hemolysis but some classes of IgG could bind complement and cause intravascular hemolysis. Anti-Kidd causes rapid, severe and extravascular hemolysis (Harmening, 2012; Kaushansky, 2016). A significant cause of DHTR is the presence of undetectable clinically significant antibodies, due to low titer which can lead to these antibodies being too weak to be detected at the time of pre-transfusion testing (Pineda et al., 1999).

Iron overload is also a blood transfusion complication that appears clear especially in chronically transfusion dependent patients because each mL of packed red blood cells contains 1mg of iron, which means each packed cell unit contains 200-250 mg of iron. This large amount of iron accumulates in the tissues of organs including the pancreas, thyroid gland, and heart leading to their failure (Kaushansky, 2016).

2.6. Alloimmunization

Alloantibodies act against foreign erythrocyte antigens after being exposed to them. This exposure could occur after blood transfusion, solid organ transplantation or pregnancy through delivery. The immune response mechanism to the formation of alloantibodies is called alloimmunization. Alloimmunization could lead to mortality or morbidity by causing hemolytic transfusion reactions. This hemolysis could cause renal failure. Patients

with alloantibody against high frequent antigens or with multiple alloantibodies could have complications related to anemia due to late in providing of compatible units for transfusion. For certain diseases, this delay could be lethal.

2.6.1. Clinical Predictors of Alloimmunization:

Many different factors influence the possibility of transfusion recipients forming erythrocyte alloantibodies, including recipient or donor/product related factors.

Alloimmunization depends on individual susceptibility to developing alloantibodies. Individuals with a high susceptibility to alloimmunization are dubbed alloantibody responders. Identifying the clinical conditions that predispose to alloimmunization is important to influence the management of a patient and lead to better understanding the etiology of transfusion reactions associated with alloimmunization. Bauer et al. identified solid malignancy, diabetes mellitus, and female gender as risk factors for alloimmunization against erythrocyte antigens but symptomatic atherosclerosis and lympho-proliferative disorders were identified as a protector against alloimmunization (Bauer, Wiersum-Osselton, Schipperus, Vandenbroucke, & Briet, 2007). Schonewille et al. reported that the possibility of the formation of alloantibodies in patients who formed a single alloantibody could be increased by 29-fold to form additional alloantibodies compared to those who had not already been alloimmunized (Schonewille, van de Watering, & Brand, 2006). One of the most important factors related to recipient impact alloimmunization is the genetic factor which is responsible for variability in Human Leukocyte Antigen (HLA). Human leukocyte antigens variability affects the ability to process and present particular peptides derived from erythrocyte antigens by class I and class II Major Histocompatibility Complex (MHC), where some types of HLA may be associated with responder phenotypes more than others (Schonewille et al., 2014).

Moreover, there are factors that impact alloimmunization that are not related to recipient patients. These factors include the non-RBC contents of transfused RBC units such as leukocytes and their remnants, platelets and their remnants, and soluble factors including cytokines. These remnants are variable among transfusion centres as a result of methodologies of blood collection and processing, lengths of holding time prior to processing, leukoreduction filter techniques and centrifugation speeds. These variables impact alloimmunization through impact recipient immune response by the ability of cells and cell remnants to induce a pro-inflammatory response driving T-cell proliferation (Danesh et al., 2014). During the storage of blood, microRNAs (miRNA) are produced in varying quantities (Kannan & Atreya, 2010). Some studies suggest that miRNAs are involved in regulating the immune response by influencing T-helper cell differentiation, which could lead to miRNAs having a potential role in alloimmunization (Baumjohann & Ansel, 2013), for which old RBC units have a higher possibility to induce alloimmunization. Antigen specificity is different for different antigens, so antigen type impacts the alloimmunization response due to differences in antigen structures. Therefore, some erythrocyte antigens are more immunogenic than others.

2.6.2. Mechanism of Erythrocyte Alloimmunization:

Erythrocyte alloimmunization is one of the immune responses against foreign antigens, alloantigens in this mechanism are erythrocyte foreign antigens. After erythrocytes are phagocytosed by macrophages, monocytes or dendritic cells, the proteins carrying the alloantigenic epitopes are processed. When antigenic epitopes are processed, these epitopes bind to the groove of HLA class II molecules and are presented to the T cell receptor of CD4 positive T cells. CD4 positive T cells then activate B cells, which are also able to recognize the antigenic epitopes, which activate B cells subsequently and produce antibodies against the corresponding epitopes. In contrast with HLA class I and class II, the

antigen-binding groove of MHC class II molecules is open at both ends while the corresponding groove on class I molecules is closed at each end. The antigens presented by MHC class II molecules are longer and consist of 15-24 amino acid residues while HLA class I molecules encompass 8–10 amino acids (Kormoczi & Mayr, 2014).

2.7. Thalassemia and Chronic Blood Transfusion

Thalassemia is defined as the reduction in the synthesis rate of one or more globin chains leading to imbalanced globin chain synthesis which leads to defective hemoglobin production. This imbalance in globin chain synthesis occurs as a result of mutations in globin genes which leads to a reduction in the rate of synthesis of the corresponding globin chains of hemoglobin. Therefore, thalassemia is classified depending on the globin gene which is affected by mutations and its corresponding globin chain.

The first description of thalassemia was offered in 1925 in Detroit by Thomas Cooley. Nowadays, thalassemia is identified as the most common hemoglobinopathy worldwide, with about 60,000 children born annually being affected with β-thalassemia (TIF, 2007) and immigrations to European countries and other countries make thalassemia as worldwide disorders.

2.7.1. Normal Hemoglobin:

Hemoglobin is a specialized intracellular erythrocyte protein with a molecular weight of 66,700 Daltons. This protein is responsible for transferring oxygen from the lungs to the tissues of the body. Each gram of hemoglobin can carry 1.34 ml of oxygen. About 33% of the volume of an erythrocyte and about 90% of a cell's dry weight are occupied by hemoglobin, with each erythrocyte containing 28-34 pg of hemoglobin. Anemia occurs as

a result of a decreased amount of hemoglobin in erythrocytes or a reduction in the erythrocyte count.

Hemoglobin synthesis begins in the pronormoblast stage but with a small amount. Most hemoglobin synthesis occurs in the development of normoblasts at the polychromatophilic normoblast stage. About 75-80% of hemoglobin is synthesized before extrusion of the nucleus and 20-25% is synthesized in reticulocytes by using residual RNA and mitochondria (McKenzie et al., 2015).

In general, the hemoglobin molecule is a tetramer consisting of four globular protein subunits, with each subunit having a hem group and globin chain, (Figure 2.1) and (Figure 2.2). The hem group consists of a tetrapyrrole ring, with ferrous iron located at its centre, (figure 2.3).

Globin chains are classified into two groups; alpha-like chains and non-alpha chains. Alpha-like chains include alpha (α) and zeta (ζ) chains. Non-alpha chains include beta (β), delta (δ), epsilon (ε) and gamma (γ) chains.

Each hemoglobin molecule consists of two identical alpha-like chains and two identical non-alpha chains. This variation in globin chains in hemoglobin molecules leads to the formation of different types of hemoglobin. Some of these types are present only in certain periods of human level of development such as hemoglobin Gower I ($\zeta_2\varepsilon_2$), Gower II ($\alpha_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$), present only in embryonic blood and absent in foetal and adult blood. Other types of hemoglobin are present in different levels of human development but with different

percentages of total hemoglobin such as hemoglobin F ($\alpha_2\gamma_2$), which is the predominant form of hemoglobin in the foetus and newborns. Moreover there is a small proportion of hemoglobin A ($\alpha_2\beta_2$) in foetal blood, though in adults hemoglobin A_1 is the predominant form with a small proportion of hemoglobin F and hemoglobin A_2 ($\alpha_2\delta_2$).

The main function of hemoglobin is to transport oxygen from the lungs to tissues, but these different types of hemoglobin have different oxygen affinity (McKenzie et al., 2015).

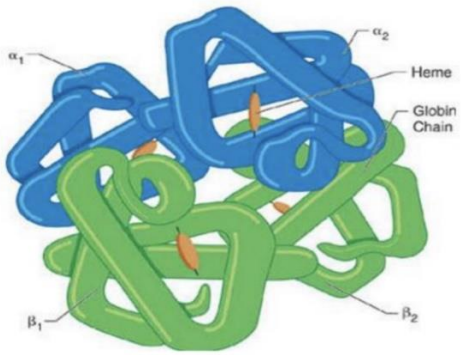

Figure 2.1: General Structure of Hemoglobin A1.

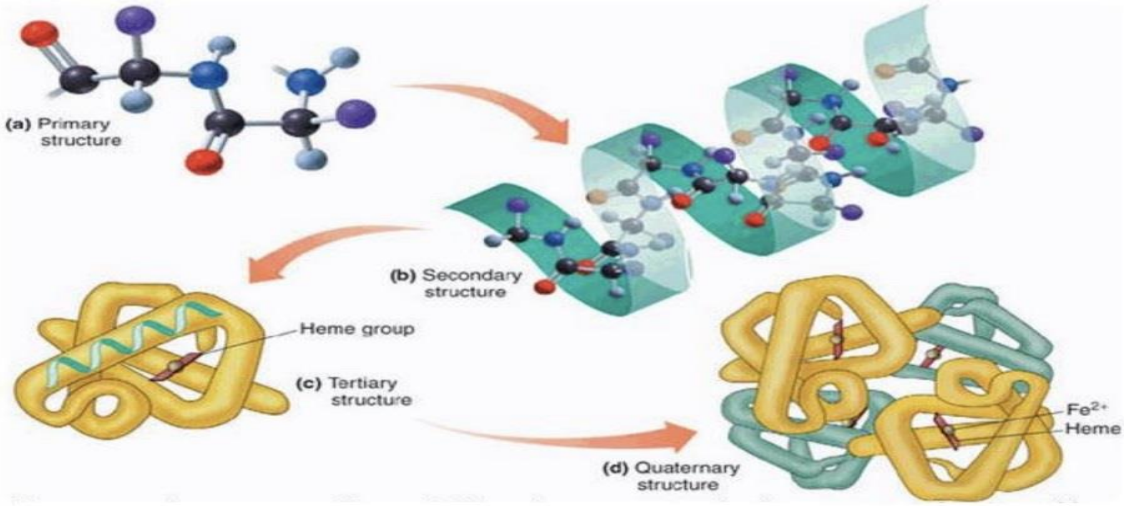

Figure 2.2: The Structure of hemoglobin: **a:** Primary structure: sequence of amino acids; **b:** Secondary structure: coiled α-helix and β-pleated sheet; **c:** Tertiary structure: folding of the molecule into a three-dimensional form; **d:** Quaternary structure: combination of four polypeptide subunits.

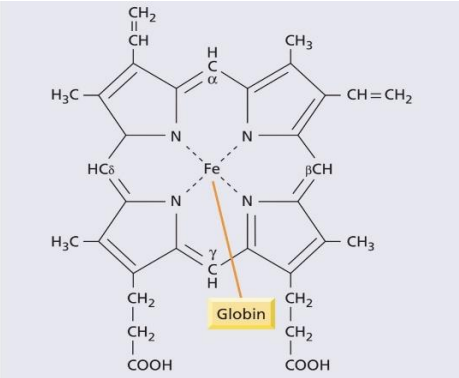

Figure 2.3: Structure of hem group

2.7.2. Thalassemia Pathophysiology:

Thalassemia is not a single disease but a group of diseases, each resulting from inherited abnormalities of globin production leading to a reduction in the rate of synthesis of one or more globin chains. The anemia present in thalassemia patients is a physiological result of imbalanced globin chain production which leads to ineffective erythropoiesis and hemolysis. In β thalassemia, there are two main forms; one is major β thalassemia as a result of the absence or decrease of β chain production and its formula as β^0-thalassemia and β^+ -thalassemia, while the other form is intermediate or minor β thalassemia which has a partial deficiency of β chain production. β-thalassemia is distributed widely in Mediterranean populations, the Middle East, parts of India and Pakistan, and throughout Southeast Asia. However, nowadays, immigrations to European countries make thalassemia as worldwide disorders. In normal hemoglobin A_1, the α chain to β chain ratio is one to one but in β thalassemia, the mutation in the β gene leads to a reduced amount of β globin chains or a total absence which leads to an excess in α chains. In major β thalassemia, this ratio is reduced to less than 0.25 (McKenzie, 2015, Kaushansky, 2016).

Excess α chains cannot form tetramers, so this excess of α chains binds to the erythrocyte membrane and leads to a decrease in erythrocyte deformability and membrane damage due to the presence of reactive oxygen species which are generated by the presence of free iron

and hemichromes in accumulated α chains. These excess α chains are the major source of anemia in β thalassemia patients: the oxidation of membrane band 3 leads to the clustering of proteins which create new antigens on the cell surface that bind to IgG and complement, leading to the destruction of IgG-complement sensitized erythrocytes in the bone marrow by binding macrophages by Fc receptors which lead to phagocytosis and the destruction of precipitate-filled erythrocytes which results in ineffective erythropoiesis and a large degree of hemolysis (McKenzie, 2015, Kaushansky, 2016).

Excess precipitation of α chains also leads to the activation of the apoptotic mechanism which causes ineffective erythropoiesis. Furthermore, circulate erythrocytes with excess precipitate α chains are removed by the spleen in the extravascular hemolysis mechanism. This dramatic reduction in β globin leads to a dramatic reduction in hemoglobin A production and leads to an increase in the production of non-β chain containing hemoglobin such as hemoglobin F and hemoglobin A_2. However, hemoglobin F has higher oxygen affinity than hemoglobin A_1 which leads to exacerbating the compromised oxygen delivery to tissues and compensates stimulation erythropoiesis present as erythroid hyperplasia which causes bone marrow expansion and thinning of calcified bone. In addition, increased erythropoiesis activity leads to an increase in iron absorption in the gut which leads to an increase in iron toxicity. Ineffective erythropoiesis in the bone marrow is accompanied by extramedullary hemopoiesis in the liver and spleen which cause hepatosplenomegaly (McKenzie et al., 2015).

At the molecular level, β thalassemia is extremely heterogeneous because there are more than 200 different mutations associated with its phenotype including deletions of the β globin gene and non-deletion mutations such as mutations that affect the transcription, processing or translation of β-globin messenger (Kaushansky, 2016).

2.8. Treatment of Thalassemia

To reduce the side effects of anemia associated with thalassemia and maintain normal developmental and growth patterns is the main goal of thalassemia treatment. Regular transfusion is the main treatment for thalassemia major patients, but nowadays, thalassemia patients can be treated by stem cell transplantation if a compatible donor is available. Moreover, there are certain novel studies that have employed gene therapy in thalassemia treatment (Kaushansky, 2016).

2.8.1. Chronic Blood Transfusion:

Patients with thalassemia major need a regular blood transfusion to reduce the side effects of anemia and allow normal developmental and growth patterns by maintaining hemoglobin levels between 9 and 10.5 g/dl (Jha R, 2014). However, these lifelong transfusions lead to transfusion-associated side effects. These transfusion-associated side effects such as the large dose of iron received by chronic transfusion lead to tissue damage as a result of iron overload, so chronic transfusion is performed with iron chelation agents such as deferoxamine to decrease the deposition of iron in the tissues. Moreover, these chronic blood transfusions increase the probability of exposure to foreign erythrocyte antigens which stimulate the immune system to form alloantibodies. These alloantibodies reduce the availability of compatible blood units for thalassemia patients. Phenotype screening and compatibility between thalassemia patients and transfused units could reduce the probability of alloimmunization (Jha R, 2014).

2.8.2. Allogeneic Hematopoietic Stem Cell Transplantation:

The most important challenge in allogeneic stem cell transplantation therapy is the availability of human leukocyte antigen (HLA) compatible donors and the prevention of the graft versus host reaction (GVHR). The main purpose of the therapy is the replacement

of the affected hematopoietic stem cells with normal hematopoietic stem cells from a compatible donor to produce normal erythrocytes. There are three sources of hematopoietic stem cells for allogeneic transplantation: bone marrow, peripheral blood and cord blood. There are advantages and disadvantages for each source. The advantages for peripheral blood stem cell transplantation include easy donation, the harvesting of high doses of stem cells and faster hematological recovery, while the disadvantages include the higher probability of acute and chronic GVHD than with other stem cells sources (Appelbaum, Forman, Negrin, & Antin, 2015).

The first allogeneic hematopoietic stem cell transplantation therapy was done in 1981 in Seattle, USA for a 14-month-old thalassemia major patient who had been transfused with 250 mL of packed RBCs. This patient received compatible allogeneic hematopoietic stem cells for transplantation from his HLA-identical sister. The treatment was completely successful (Thomas et al., 1982).

In the same period, in 1984, another hematopoietic stem cell transplantation therapy was undertaken in Pesaro in Italy for a 14-year-old thalassemia patient who had received 150 mL packed RBC transfusions (Lucarelli et al., 1984). This patient received compatible allogeneic hematopoietic stem cells for transplantation from his HLA-identical brother. This patient rejected the graft and was the first of an extensive series of transplants for thalassemia (Appelbaum, Forman, Negrin, & Antin, 2015).

2.8.3. Gene Therapy:

Because β thalassemia is one of several single gene defect diseases worldwide, gene therapy could be used to express the normal β globin gene. This type of therapy depends on the autologous transfer of genetically modified hematopoietic stem cells. Many efforts have been made but with very limited success so far. The first successful trial was reported

in 2007 and the result was published after 33 months post-gene transfer (Cavazzana-Calvo et al., 2010). The trial was done in an adult patient with severe β^E/β^0-thalassaemia who was dependent on monthly transfusions and received hematopoietic stem cell lentiviral β globin gene therapy. After 21 months, 100% of hemoglobin A came from the modified cells and the patient was transfusion free. The patient subsequently experienced a good quality of life with a stable hemoglobin level of between 9 and 10 g/dl, and was transfusion-free and cancer-free for up to 7 years (Nienhuis, 2013). For this type of therapy there are still more trials needed (Bank, Dorazio, & Leboulch, 2005).

2.9. Related study

There is a variation in the results according to the studies of the frequency of alloimmunization among transfusion-dependent thalassemia patients. However, there is no data available for the frequency among transfusion-dependent thalassemia patients in the State of Palestine.

Previous studies have shown very high frequencies of alloimmunization among transfusion-dependent thalassemia patients ranging from 22% to 42%. The highest frequency of alloimmunization of 42.5% was presented in the province of Alexandria in Egypt which was reported by Obaid et al. in 2015. In a study by Wang et al. in Taiwan in 2006, 37% of thalassemia major patients were found to carry alloantibodies.

In another study by Ameen et al. in 2003 among transfusion dependent Arab thalassemia patients in Kuwait, the frequency of alloimmunization was 30%. Hussein et al. reported in a study done in Egypt in which 22.8% of the transfusion-dependent thalassemia patients have at least one alloantibody. In Greece, a frequency of alloimmunization of 22.6% was reported by Spanos et al. in 1990 (Ameen et al., 2003; Hussein, Desooky, Rihan, & Kamal,

2014; Obaid, Abo El-Nazar, Ghanem, El-Hadidi, & Mersal, 2015; TH Spanos et al., 1990; Wang et al., 2006).

There are other studies reporting a low frequency of alloimmunization among transfusion-dependent thalassemia patients. Pahuja et al. reported the frequency of alloimmunization among Indian origin thalassemia patients to be 3.8% (Pahuja, Pujani, Gupta, Chandra, & Jain, 2010).

Seferi et al. reported that 11.8% of transfusion dependent Albanian thalassemia patients were alloimmunized, 10.1% of patients were already alloimmunized before the study and only 1.7% of patients were alloimmunized after applying a strict Rh and Kell matching policy (Seferi et al., 2015). In Egypt, different studies were done to identify the frequency of alloimmunization among transfusion-dependent thalassemia patients in different regions (Abdelrazik et al., 2016; el-Danasoury, Eissa, Abdo, & Elalfy, 2012; Hussein et al., 2014; Obaid et al., 2015). Abdelrazik et al. and Obaid et al. reported the frequency of alloimmunization among transfusion-dependent thalassemia patients in Fayoum province and Alexandria provinces respectively to be 8% and 42.5% (Abdelrazik et al., 2016; Obaid et al., 2015).

Several studies have also been carried out in Iran. Davari et al., Amin et al., and Karimi et al. performed studies in the north-west, south-east and southern regions of Iran and reported that 16.3%, 17.9% and 5.3% of the transfusion-dependent thalassemia patients had been alloimmunized respectively. A descriptive study was also undertaken in Iran by Azarkeivan et al. in thalassemia centres (two were paediatric centres and two were adult centres) to identify the frequency of alloimmunization which was 7.7% of paediatric and 14.4% of adult had alloantibodies (Amin, 2013; Azarkeivan et al., 2011; Davari & Soltanpour, 2016; Karimi, Nikrooz, Kashef, Jamalian, & Davatolhagh, 2007).

In 1985, Sirchia et al. reported that 5.2% of Italian transfusion-dependent thalassemia patients had been alloimmunized (Sirchia et al., 1985). Guirat-Dhouib et al. reported that 7.7% of Tunisian transfusion-dependent thalassemia patients had at least one alloantibody (Guirat-Dhouib et al., 2011). Abdel Gader et al. reported that 22.06% of multi-transfused patients had been alloimmunized in Saudi Arabia (Gader, Al Ghumlas, & Al-Momen, 2008). In india, in the Jammu region, Dogra et al. reported that 8.5% of transfusion-dependent thalassemia child patients had been alloimmunized (Dogra, Sidhu, Kapoor, & Kumar, 2015). Haslina et al. reported that 8.6% of Malay transfusion-dependent thalassemia patients had alloantibodies (Haslina, Ariffin, Hayati, & Rosline, 2006). In Pakistan, Zaidi et al. reported that 8.6% of thalassemia transfusion-dependent patients had been alloimmunized (Zaidi et al., 2015).

In the previous mentioned studies, several independent factors were studied including gender, status of spleen, number of transfusions, blood group (ABO/Rh-D) and age of starting transfusion therapy. The most important factor however was found to be the heterogeneity between the erythrocyte antigens of the donor and recipient.

Splenectomy was reported as a risk factor for alloimmunization in several studies. Singer and Hussein suggest that Splenectomy is a risk factor for alloimmunization, that stimulate the immune system to produce alloantibodies due to predominantly abnormal RBC deformability (Singer et al., 2000, Hussein et al., 2014). While other studies showed that there is no association between alloimmunization and splenectomy (Al-Mousawi, Al-Allawi, & Alnaqshabandi, 2015; Karimi, Nikrooz, Kashef, Jamalian, & Davatolhagh, 2007; Pahuja, Pujani, Gupta, Chandra, & Jain, 2010; Sirchia et al., 1985).

Furthermore, Singer and Al-Mousawi suggested that alloimmunization could be affected by a patient's age at starting transfusion and that transfusion at an early age (less than 1-3

years old) may offer some immune tolerance and protection against alloimmunization in transfusion-dependent thalassemia patients (Al-Mousawi, Al-Allawi, & Alnaqshabandi, 2015; Singer et al., 2000). Other studies, reported that there is no significant association between alloimmunization and age of starting transfusion (Amin, 2013; Karimi, Nikrooz, Kashef, Jamalian, & Davatolhagh, 2007).

Singer and Al-Mousawi suggested that there is no association between alloimmunization and the number of transfused units, though Karimi et al., Dogra et al. and Hussein et al. all suggested that there is significant correlation with the number of blood units.

Female gender has been known as a risk factor for alloimmunization (Bauer et al., 2007). Moreover, Dogra et al. reported no significant association between alloimmunization and gender (Dogra et al., 2015). In fact, males had a higher alloimmunization rate in Hussein's study (Hussein et al., 2014).

Al-Mousawi et al. reported that there is significant association between alloimmunization and the absence of the Rh-D antigen (Al-Mousawi, Al-Allawi, & Alnaqshabandi, 2015).

Chapter Three

3. Materials and Methods

3.1.Study design

A cross-sectional study was done by convenient sampling from the transfusion-dependent thalassemia patient population in the middle and southern regions of the West Bank. This was done at the transfusion centres in the Palestinian Central Blood Bank in Ramallah, Jericho Hospital in Jericho and Hebron Hospital (Alia) in Hebron.

Depending on recently updated data from Thalassemia Patients Friends Palestinian Society, in the West Bank there are 500 transfusion-dependent thalassemia patients, with 153 patients (30.6%) in the investigated regions of the West Bank receiving blood transfusion in the centres mentioned.

The study in the northern region of the West Bank was done in the first phase of our study in June 2016. The frequency of alloimmunization among transfusion-dependent thalassemia patients in northern region of the West Bank was 14% anti-D followed by anti-K were the most frequent alloantibodies.

3.2.Study Population and Sample Size

Transfusion-dependent thalassemia patients in the middle and southern regions of the West Bank who met the inclusion criteria were the study population.

The sample size was calculated using the Raosoft® sample size calculator (http://www.raosoft.com/samplesize.html). The Raosoft® software indicated that the study had a 5% accepted margin of error, a 95% confidence level, and a 17% response distribution with the sample size being 101 transfusion-dependent thalassemia patients (male, n=52; female, n=49). Thirty-five patients (34.6%) were from the Palestinian Central Blood Bank, 5 patients (4.95%) were from the Jericho Hospital and 61 patients (60.4%) were from the Hebron Hospital. The distribution of the sample is illustrated in (Table 3.1).

3.3.Inclusion and Exclusion Criteria

Inclusion criteria:

1. Transfusion-dependent thalassemia patients from south or middle of the West Bank.
2. Any age
3. Any gender
4. Received at least ten transfusions.
5. Last transfusion occur at least before 2 weeks

Exclusion criteria:

1. Transfusion-dependent thalassemia patients from north of the West Bank.
2. Received less than ten transfusions.
3. Last transfusion occur in period less than 2 weeks.

3.4.Ethical Considerations

The study was ethically approved by Institutional Review Board (IRB) at Al-Quds University Ref No: 5/REC/2017 (Appendix 3) and by the Palestinian Ministry of Health (PMOH).

A brief explanation was given to the patients or their guardians. Then, the patients were asked to sign a consent form (Appendix 4) to approve their participation and the collection of blood samples from them.

Table 3.1: Distribution of sample in terms of transfusion centres.

Governorates	Transfusion centre	Population (%)	Sample Size (%)
Jericho	Jericho Hospital	6 (3.9%)	5 (4.95%)
Ramallah	Central Blood Bank	54 (35.3%)	35 (34.65%)
Bethlehem	Central Blood Bank	1 (0.7%)	---
Hebron	Hebron Hospital	92 (60.1%)	61 (60.4)
Total		153 (100%)	101 (100%)

3.5. Data collection

A small questionnaire (Appendix 5) was administrated –a face to face interview with the patients- to collect data such as age, gender, status of the spleen, age of starting transfusion and frequency of transfusion were obtained directly from the participants (or their legal guardians) or from the records of Palestinian Ministry of Health (PMOH).

3.6. Laboratory Investigations

About ten ml of blood was collected from each participant: 5ml in an ethylene di-amine tetra-acetic acid (EDTA) tube and 5ml in a plain tube. Whole blood in EDTA tubes were stored in a refrigerator at 4^0 C and serum from plain tubes was stored at -20^0 C until the tests were performed in the hospital transfusion centres. After sample collection was completed, all samples were transferred to the An-Najah National University Hospital (NNUH) blood bank for testing.

3.6.1. ABO and Rh-D Identification:

To confirm the participants' blood group from the PMOH data, blood grouping (ABO & Rh-D) was performed by two methods: reverse and forward blood grouping. Forward and reverse blood grouping were done by micro-tube gel methods (ID card-Diaclon ABO/D+Reverse grouping, Diamed ID, Switzerland). Commercial A and B cell suspensions (ID-Diacell ABO A_1-B, Diamed ID, Switzerland) were used for reverse blood grouping.

A cell suspensions (5%) were prepared from EDTA whole blood for each participant by adding 50µl of whole blood to 1mL of low ionic strength saline (LISS) (ID-Diluents II, Diamed ID, Switzerland). These suspensions were used for forward blood grouping. A volume of 12.5µl of the cell suspension was added to micro-tube 1 which contained anti-A, 12.5µl of the cell suspension was added to micro-tube 2 which contained anti-B and 12.5µl of the cell suspension was added to micro-tube 3 which contained anti-D for forward blood grouping, and 12.5µl of the cell suspension was added to micro-tube 4 which is the control micro-tube.

For reverse blood grouping, 50µl of each participant's serum were added to micro-tube 5 and 6, 50µl of commercial A-cells were added to micro-tube 5, and 50µl of commercial B-cells were added to micro-tube 6.

After preparing the micro-tube gel cards for the participants, they were centrifuged for 10 minutes.

The result of forward and reverse blood grouping were done automatically using a WADiana® cc-702 compact analyser (Diagnostic Grifols, Spain) at the NNUH blood bank. As a control, three known blood group samples (A positive, B positive and O negative) were performed at each patch.

3.6.2. Alloantibody Screening:

The indirect Coombs test was done to detect the presence of alloantibodies by using micro-tube gel cards with poly-specific antihuman globulin (anti-IgG) and an anti-C3d complement. In addition, three sets of commercial group O human RBCs that were typed for clinically significant antigens as well as rare antigens (ID-Diacell I-II-II, Diamed ID, Switzerland) were used in the indirect Coombs test for alloantibody screening.

A volume of 50µl of ID-Diacell I was added to micro-tube 1, 50µl of ID-Diacell II was added to micro-tube 2, 50µl of ID-Diacell III was added to micro-tube 3, and 25µl of the participant's serum was added to each micro-tube used. After preparing the micro-tube gel cards for the participants, these cards were incubated for 15 minutes at 37^0 C in an ID-incubator. Finally, they were centrifuged for 10 minutes.

Two known samples were used as a control, Positive and negative samples were done at each patch.

Alloantibody screening and reading of the results were done automatically using a WADiana® cc-702 compact analyser (Diagnostic Grifols, Spain) at the NNUH blood bank.

3.6.3. Alloantibody Identification:

The indirect Coombs test was done to identify the alloantibodies which were detected in the antibody screening step. Micro-tube gel cards with poly-specific antihuman globulin (anti-IgG) and an anti-C3d complement were used, however 11 sets of commercial group O human RBCs that had been typed for clinically significant antigens as well as rare antigens (ID-DiaPanel, Diamed ID, Switzerland) were used in the indirect Coombs test for alloantibody identification. In two micro-tube gel cards, 50µl from each ID-DiaPanel set (1-11ID-DiaPanel) was added to separate micro-tubes (micro-tubes 1-6 in the first gel card and 7-11 in the second gel card). Then, 25µl of the participant's serum was added to micro-tube 1-11. After preparing the micro-tube gel cards for the participants, they were incubated for 15 minutes at 37^0 C in an ID-incubator. Finally, they were centrifuged for 10 minutes.

Alloantibody identification process and the reading of the results were done automatically using an WADiana® cc-702 compact analyser (Diagnostic Grifols, Spain) at the NNUH blood bank. A known sample, positive for anti-D was used at each patch as a control.

3.7.Statistical Analysis

Statistical analysis was performed by using the Statistical Package for the Social Science (SPSS) software (version 18.0, IBM, USA). It included descriptive statistics, frequency distribution, mean, and standard deviation calculations. Comparative studies were performed by using Chi-square test. Statistically significance was considered as a *P* value <0.05.

Chapter Four

4. Results

4.1.Participants Characteristics

In this research study, a total of 101 thalassemia patients were included. Eighty-eight participants (87.1%) were thalassemia major and 13 participants (12.9%) were thalassemia intermedia (Table 4.1). Forty-four participants (49%) were females and 52 participants (51%) were males, resulting in a female to male ratio of 0.94 (Figure 4.1).

Table 4.1: Distribution of the thalassemia phynotypes of participants

Diagnosis	Number of participants	Percentage
Thalassemia Major	88	87.1%
Thalassemia Intermedia	13	12.9%

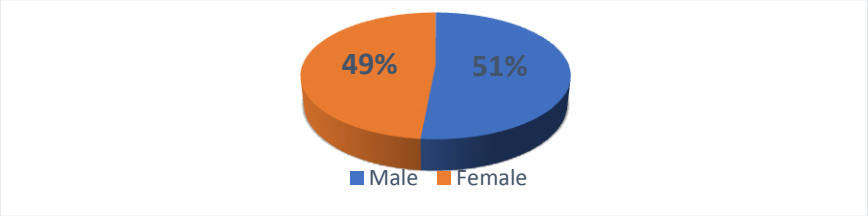

Figure 4.1: Distribution of gender of participants.

Of the 101 participants, 61 were from Hebron (60.4%), 35 were from Ramallah (34.7%) and 5 were from Jericho (4.9%).

The 101 patients were grouped to different group ages. Twenty-two of the 101 participants (21.8%) were less than 10 years old, 39 (38.6%) were between 10 and 20 years old, 27 participants (26.7%) were between 20 and 30 years old and 13 (12.9%) were more than 30

years old. The age of the participants ranged from 3 to 53 years old, the mean was 19 years, the median was 18 years and standard deviation was 9.8 years.

In this study, 44 participants (43.6%) had A blood group, 29 (28.7%) had O blood group, 18 (17.8%) had B blood group, and 10 (9.9%) had AB blood group (Table 4.2).

Regarding to the D antigen in the Rh-system, 89 participants (88.1%) had positive Rh-D antigen and 12 participants (11.9%) had negative Rh-D antigen (Table 4.3).

Table 4.2: Distribution of ABO among participants

ABO / Gender	A	B	O	AB
Male	19	10	16	7
Female	25	8	13	3
Total	44	18	29	10

Table 4.3: Distribution of Rh-D antigen among participants

Rh-D / Gender	Positive	Negative
Male	49	3
Female	40	9
Total	89	12

All the participants in this research received regular transfusions with post-storage leukoreduced packed red blood cells and the transfusion mean was 251, while the transfusions median was 208 with a range from 16 to 676. Finally, the standard deviation was 165. (Figure 4.2) summarize the transfusion number for the participants in this research.

Forty-eight participants (47.5%) in this study were splenectomised (Figure 4.3).

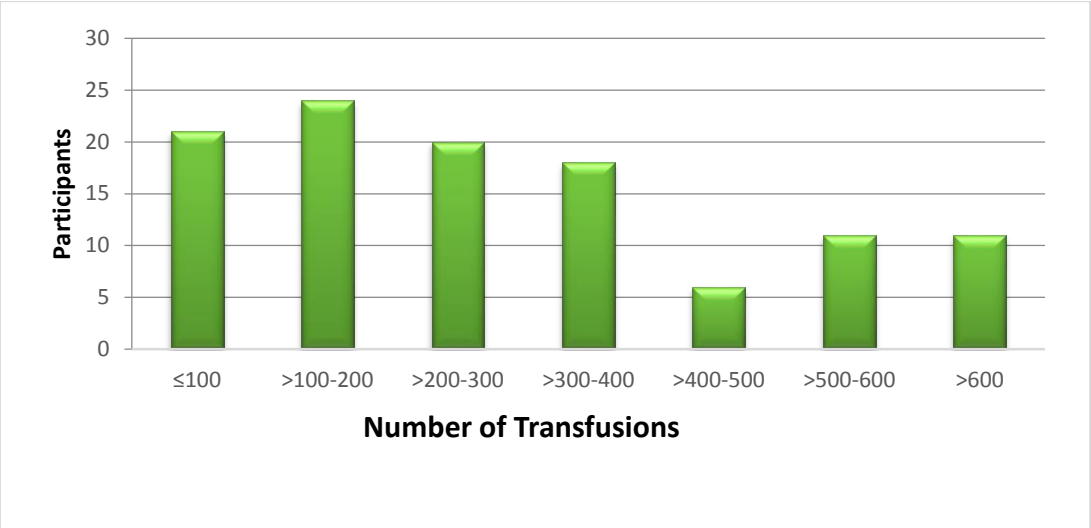

Figure 4.2: Number of transfusions of participants.

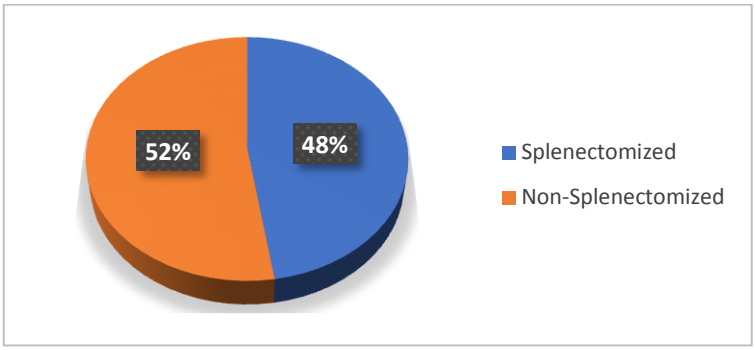

Figure 4.3: Distribution of Status of spleen.

4.2.Antibody Screening

In this research, 11 participants (10.9%) developed at least one alloantibody, ten of them were diagnosed with β-thalassemia major and one of them was diagnosed with β-thalassemia intermidia. Among the participants with alloantibodies, the mean age was 22 years with a ranging from 12 to 35 years, with eight participants being female and three being male.

Eight of 11 alloimmunized participants were splenectomized. Among the eight splenectomized participants with alloantibodies, 5 were female and 3 were male. This

means that all of the alloimmunized male participants in this research were splenectomized. Appendix 6 summarizes the characteristics of alloimmunized participants.

Among the alloimmunized participants, the mean number of transfusions was 388 with a range from 208 to 589 transfusions.

Among the 11 participants with alloantibodies, 3 (27.3%) had A-Positive blood group, 2 (18.2%) had A-Negative blood group, 2 (18.2%) had B-Positive blood group, one (9.1%) had O-Positive blood group, one (9.1 %) had O-Negative blood group and 2 (18.2%) had AB-Positive blood group.

4.3.Antibody Identification

Among 11 participants with alloantibodies, seven (63.6%) had developed single alloantibodies and four (36.4%) had developed more than one alloantibody (three of them had two alloantibodies and one had three alloantibodies). These 11 alloimmunized participants developed 6 different significant alloantibodies: three antibodies (50%) were against antigens belonging to the Rh-system (anti-E, anti-C and anti-D), two antibodies (33.3%) were against antigens belonging to the Kell-system (anti-K and antiKp^a) and one antibody was against antigen belonging to the Kidd-system (anti-Jk^a), (Table 4.4).

Anti-E was found in 5 cases; in two cases as a single alloantibody (case-1 and case-3) and as multiple alloantibodies in three cases(caase-4, case-8 andcase-11).

Anti-K was found in 3 cases, in one case as a single alloantibody (case-6) and as multiple alloantibodies (case-4 and case11).

Moreover, anti-Jk^a was found in 3 cases, one as a single alloantibody (case-10) and two as multiple alloantibodies (case-8 and case-11).

Anti-D was found in 2 cases who are negative for D antigen, in one case as a single alloantibody (case-5). Anti-C was found in 2 cases, in one case as a single alloantibody (case-7). In case-9, anti-D and anti-C were present as multiple alloantibodies.

Anti-Kp^a was present in one case (case-2), (Appendix 6).

Table 4.4: Alloantibodies detected among study participants

Blood Group systems	Abs	Number	Percentage
Rh-system	Anti-D	2	12.5%
	Anti-C	2	12.5%
	Anti-E	5	31.25%
Kell-system	Anti-K	3	18.75%
	Anti-Kp^a	1	6.25%
Kidd-system	Anti-Jk^a	3	18.75%
Total		16	100%

4.4. Association between Characteristics of Participants and Alloimmunization

4.4.1. Governorates (Blood Transfusion Centres):

This research study was done in three transfusion centres in the middle and southern areas of the West Bank. Most alloimmunized participants were from Hebron (10/11; 90.9%), just one alloimmunized participant was from Ramallah and no participants were detected from Jericho (Table 4.5).

There was no significant association between alloimmunization and transfusion centres (P-value = 0.089 > 0.05).

Table 4.5: Alloimmunized participants according to governorate with P-value

Transfusion centre	Number of alloimmunized patients	Percentage out of 101	P-value
Jericho	0/5	0 %	0.089
Ramallah	1/35	0.99 %	
Hebron	10/61	9.9 %	
Total	11/101	10.89%	

4.4.2. Gender:

Of the 11 alloimmunized participants, 8 were females (72.7%) and 3 were males (27.3%) (Figure 4.4).

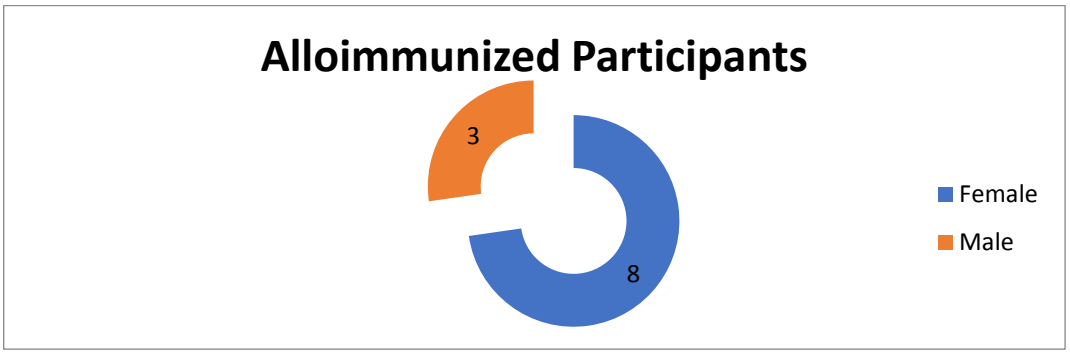

Figure 4.4: Distribution of alloimmunized participants according to gender.

This research study involved 49 female and 52 male participants, with 8 (16.3%) and 3 of these groups being alloimmunized (5.8%) respectively (Table 4.6).

Table 4.6: Association between alloimmunization and gender

	Alloimmunized	Non-alloimmunized	Total	P-value
Male	3 (5.8%)	49 (94.2%)	52	0.089
Female	8 (16.3%)	41(83.7)	49	
Total	11	90	101	

There was no significant association between alloimmunization and gender (P-value = 0.089 > 0.05).

4.4.3. Age:

The age of the participants was one of the variables in our study with a range from 3 to 53 years old, a mean of 19 years, a median of 18 years and standard deviation of 9.8 years.

All participants were categorized in different age groups as presented in (Table 4.7).

There was no significant association found between alloimmunization and the age of participants (P-value = 0.248 > 0.05).

Table 4.7: Association between alloimmunization and age

Intervals	Number of Participants	Alloimmunized	P-value
≤ 10 years	22 (21.8%)	0	0.248
>10-20 years	39 (38.6%)	4	
>20-30 years	27 (26.7%)	6	
>30-40 years	10 (9.9%)	1	
>40-50 years	2 (2%)	0	
>50-60 years	1 (1%)	0	

4.4.4. Status of Spleen:

Of the 101 participants in this research study, 48 were splenectomized (47.5%), of whom, 8 were Alloimmunized (16.7%). (Figure 4.5).

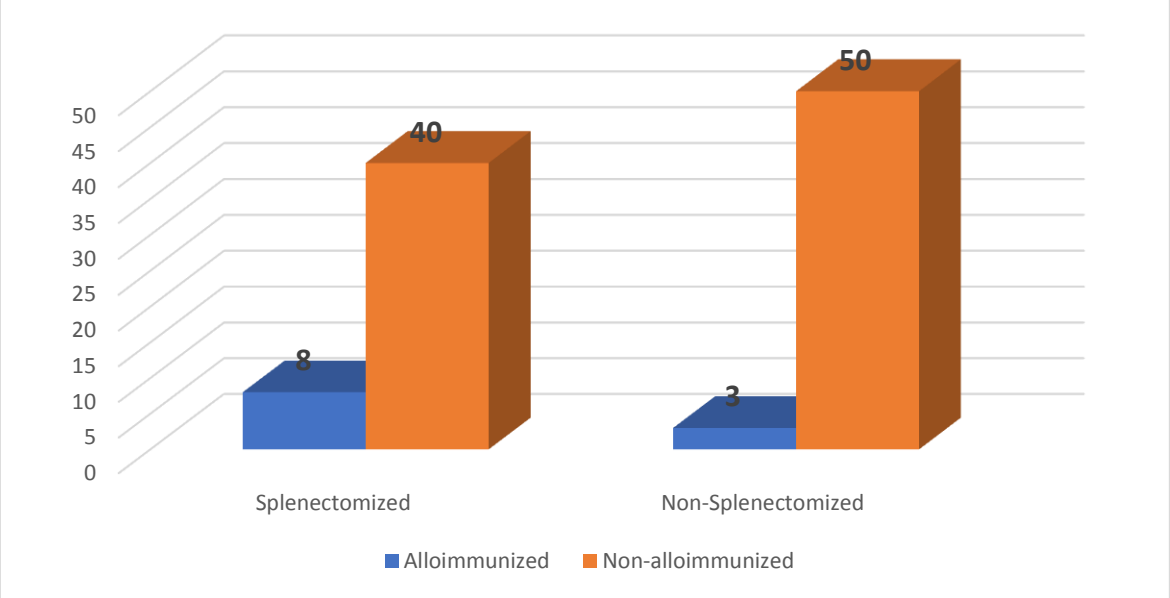

Figure 4.5: Alloimmunized participants according to status of spleen.

There was no significant association between alloimmunization and status of spleen (P-value = 0.076 > 0.05).

4.4.5. Age of first blood transfusion:

The population in this research study is thalassemia patients; as a result of having received transfusion therapy, most of the participants started blood transfusion before the age of 2 years old. However, there were some exceptions, such as when the patient was diagnosed as thalassemia intermedia. Therefore, the starting transfusion age was classified into five categories as shown in (Figure 4.6).

No significant association was found between alloimmunization and age of starting transfusion (P-value = 0.802 > 0.05).

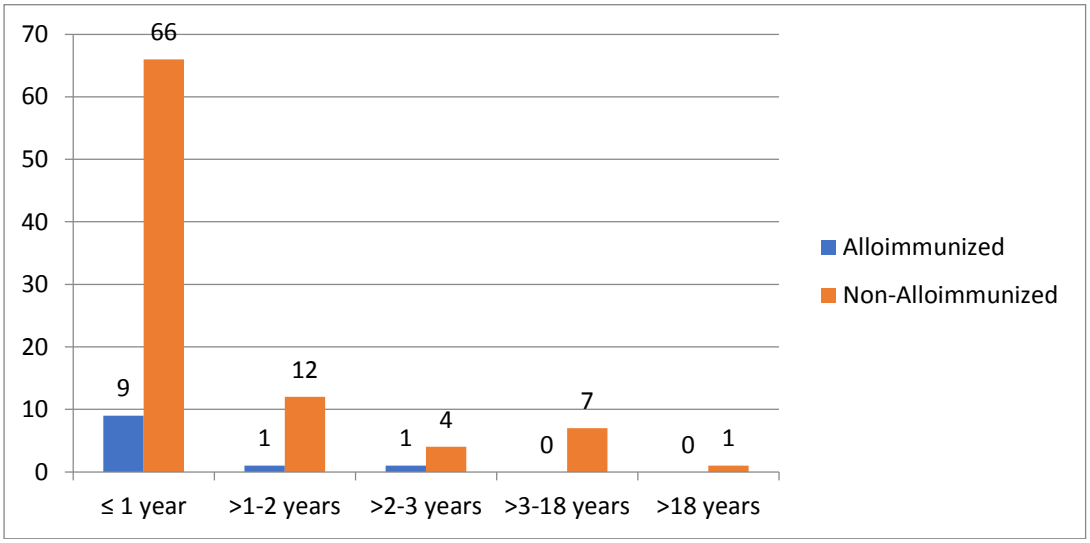

Figure 4.6: Alloimmunized participants according to age of starting transfusion.

4.4.6. Frequency of transfusion:

The frequency of transfusion was classified into three periods as shown in (Table 4.8). Most participants received at least one unit of blood per month.

Table 4.8: Association between alloimmunization and frequency of transfusion

Frequency of Transfusion	Number of Participants	Alloimmunized	P-value
≥ 1 unit/month	80 (79.2%)	10	0.575
< 1 unit/month	18 (17.8%)	1	
< 1 units/2 month	3 (2.9%)	0	

No significant association was found between alloimmunization and frequency of transfusion (P-value = 0.575 > 0.05).

4.4.7. Number of transfusion:

In this research study, the number of transfusions range from 16 to 676, the mean was 251, the median was 208 and the standard deviation was 165.

Table 4.9: Association between alloimmunization and number of transfusions

Intervals	Number of Participants (%)	Alloimmunized	P-value
≤100 Transfusions	21 (20.8%)	0	0.069
>100-200 Transfusions	24 (23.8%)	1	
>200-300 Transfusions	20 (19.8%)	3	
>300-400 Transfusions	18 (17.8%)	2	
>400-500 Transfusions	6 (5.9%)	1	
>500-600 Transfusions	11 (10.9%)	4	
>600 Transfusions	1 (1%)	0	

No significant association was found between alloimmunization and number of transfusions (P-value = 0.069 > 0.05) (Table 4.9).

4.4.8. ABO/Rh-D blood group:

In this research study, the ABO blood group system and Rh-D were independent variables. There was no significant association between alloimmunization and the ABO blood group (P-value = 0.193 > 0.05) (Table 4.10). Also, there was no significant association between alloimmunization and the status of the D antigen (P-value = 0.095 > 0.05) (Table 4.11).

Table 4.10: Association between alloimmunization and ABO blood group

ABO	Number of Participants (%)	Alloimmunized	P-value
A	44	5	0.719
B	18	2	
O	29	2	
AB	10	2	

Table 4.11: Association between alloimmunization and status of D antigen

Rh-D	Number of Participants (%)	Alloimmunized	P-value
Positive	89	8	0.095
Negative	12	3	

Chapter Five

5. Discussion

Thalassemia is a lifelong transfusion dependent disease. Continuous blood transfusion can cause alloimmunization against RBC antigens and complicate further treatment in these patients. The purpose of this study was to determine the frequency of RBC alloantibodies, the types of these antibodies and the factors that affect alloimmunization in these patients.

In Palestine, thalassemia patients are treated by blood transfusion. However, blood transfusions are a double-edged sword; on one hand, they are lifesaving, while on the other hand, their complications are harmful.

Thalassemia is reported to be common in the Mediterranean region, equatorial or near-equatorial regions of the Arabian Peninsula, India and southern China (Jha R, 2014).

This is the first study to have investigated the frequency of alloimmunization among transfusion-dependent thalassemia patients in Palestine. The prevalence of alloantibodies among transfusion-dependent thalassemia patients in this study was 10.9%. A similar alloimmunization rate was found by Seferi et al. in an Albanian population (11.8%) (Seferi et al., 2015). While rates were found in Pakistan to be 8.6% (Zaidi et al., 2015) and in Egypt to be 8% (Abdelrazik et al., 2016).

A higher alloimmunization incidence was reported by Singer et al. in a study done in transfusion-dependent thalassemia patients of predominantly Asian descent (22%) (Singer

et al., 2000). This higher incidence was due to a heterogeneity between donor and recipients, 75% of alloimmunized patients were Asian and 85% of blood donors in local blood centres were white (Singer et al., 2000). Moreover, a higher incidence was reported by Wang et al. in Taiwan (37%), Ameen et al. in the Arab population in Kuwait (30%), Egyptian population by Hussein et al. (30%) and Spanos et al. in Greece (22.8%) (Ameen et al., 2003; Hussein et al., 2014; T. Spanos et al., 1990; Wang et al., 2006). The common cause of these higher incidence rates of alloimmunization could be due to heterogeneity between donors and blood recipients.

Lower rates of alloimmunization were reported in Southern Iran (5.3 %) (Karimi et al., 2007), Italy (5.2%) (Sirchia et al., 1985), Iraq (5.1%) (Al-Mousawi et al., 2015) and India (3.8%) (Pahuja et al., 2010).

In this study, 56.3% of alloantibodies were against antigens from the Rh-system and 25% were against antigens from the Kell-system. The most common alloantibody was anti-E (31.25%) followed by anti-K (18.75%) and anti Jk^a (18.75%). This finding is similar to studies done in Egypt, India, Greece, Italy and Pakistan, which reported that alloantibodies which belong to the Rh-system were the most common followed by the Kell system (Hussein et al., 2014; Pahuja et al., 2010; Sirchia et al., 1985; T. Spanos et al., 1990; Zaidi et al., 2015).

In a study done by Ameen et al., it was found that alloantibodies against antigens from the Kell-system are the most common alloantibodies followed by those against the Rh-system in alloimmunized transfusion-dependent Arab thalassemia patients in Kuwait, where anti-K was the most common followed by anti-E (Ameen et al., 2003). Moreover, Karimi et al. and Davari in Iran reported that alloantibodies belonging to the Kell-system were the most

common alloantibodies (Davari & Soltanpour, 2016; Karimi et al., 2007). A lower anti-K frequency of 0.53% was reported in Fayoum, Egypt. (Abdelrazik et al., 2016).

The differences in alloimmunization rates are attributed to at least three main contributing factors as reported by Singer; the antigenicity difference between blood donors and recipients, the immune status of blood recipients and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system (Singer et al., 2000).

The homogeneity of the Palestinian population could lead to an alloimmunization rate that is not as high as that found in other heterogeneous populations such as the Kuwaiti and Asian populations. This could also be due to the fact that all thalassemia patients in Palestine receiving post-storage leukoreduced blood. Hussein et al. suggested that leukoreduced blood reduces the alloimmunization rate (Hussein et al., 2014). Furthermore, matching phenotyping for the E antigen and K antigen as well as ABO/D antigens could lead to a reduction in the alloimmunization rate in Palestine. Singer proved that matching for the Rh-system and K antigens appears to be effective in preventing alloimmunization (Singer et al., 2000).

We reported that there is no significant association between alloimmunization and Splenectomy. Furthermore, Sirchia et al. in Italy, Karimi et al. in Southern Iran, Pahuja et al. in India and Al-Mousawi et al. in Iraq reported that there is no association between alloimmunization and splenectomy (Al-Mousawi et al., 2015; Karimi et al., 2007; Pahuja et al., 2010; Sirchia et al., 1985). However, Singer and Hussein suggest that the absence of a spleen stimulates the immune system to produce alloantibodies due to predominantly abnormal RBC deformability (Hussein et al., 2014; Singer et al., 2000).

Moreover, Singer and Al-Mousawi et al. suggested that alloimmunization could be affected by a patient's age at first blood transfusion and that transfusion at an early age

(less than 1-3 years old) may offer some immune tolerance and protection against alloimmunization in transfusion-dependent thalassemia patients (Al-Mousawi et al., 2015; Hussein et al., 2014; Singer et al., 2000). However, Karimi and Amin et al. reported that there is no significant association between alloimmunization and age of starting transfusion and that there is no significant difference in the effect of alloimmunization between groups who received blood before and after 1 year old (Amin, 2013; Karimi et al., 2007). In addition, we reported that there is no significant association between alloimmunization and age of first blood transfusion.

Moreover, we reported that there is no significant association between alloimmunization and number of transfusions, Also, Singer and Al-Mousawi et al. suggested that there is no association between alloimmunization and the number of transfused units, though Karimi et al., Dogra et al. and Hussein et al. all suggested that there is significant correlation with the number of blood units (Al-Mousawi et al., 2015; Dogra et al., 2015; Hussein et al., 2014; Karimi et al., 2007; Singer et al., 2000).

Female gender has been known as a risk factor for alloimmunization (Bauer et al., 2007); but we reported that there is no significant association between alloimmunization and gender. Moreover, Dogra et al. reported no significant association between alloimmunization and gender (Dogra et al., 2015). In fact, males had a higher alloimmunization rate in Hussein's study (Hussein et al., 2014).

We reported in the present study that there is no significant association between alloimmunization and ABO blood Group and Rh-D, though Al-Mousawi et al. reported that there is significant association between alloimmunization and the absence of the Rh-D antigen (Al-Mousawi et al., 2015).

Chapter Six

6. Summary

6.1.Conclusions

Alloimmunization leads to an increase in blood transfusion complications and a reduction in the availability of compatible blood units. This serious complication is due to the presence of alloantibodies in chronically transfusion-dependent patients such as thalassemia patients.

Antibodies against antigens belonging to the Rh-system and Kell-system are the most common alloantibodies in transfusion-dependent thalassemia patients in the middle and southern areas of the West Bank. Among the 16 significant alloantibodies identified in this study, anti-E are the most common alloantibody, followed by Anti-K, Anti-Jk^a, Anti-C and Anti-D.

We found no significant association between alloimmunization and several factors such as gender, splenectomy status, ABO blood group, status of Rh-D antigen, number of transfusions and age of starting transfusion.

6.2.Limitations

Clinical data were collected from a computerized system in the Palestinian Ministry of Health, but some data such as age of first blood transfusion, transfusion frequency and

number of transfused units were unavailable. Therefore, age of starting transfusion and transfusion frequency were estimated by patients or guardians and the number of transfused units was estimated by calculations from the age of starting transfusion and transfusion frequency.

6.3.Recommendations

In order to reduce alloimmunization in these patients, a policy to perform extended red cell phenotyping and issuing antigen-matched blood should be adopted. This antigen-matching should be carried out at least for K and E antigens in addition to ABO/D matching.

Moreover, a policy to transfuse pre-storage leukoreduced blood units for transfusion-dependent patients should be adopted.

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Appendices

Appendix 1: **Blood group systems- Part 1** (ISBT, 2018)

No.	System name	System symbol	Gene name(s)*	Number of antigens	Chromosomal location	CD numbers
001	ABO	ABO	*ABO*	4	9q34.2	
002	MNS	MNS	*GYPA, GYPB, (GYPE)*	49	4q31.21	CD235a CD235b
003	P1PK	P1PK	*A4GALT*	3	22q13.2	CD77
004	Rh	RH	*RHD, RHCE*	55	1p36.11	CD240
005	Lutheran	LU	*BCAM*	25	19q13.2	CD239
006	Kell	KEL	*KEL*	36	7q33	CD238
007	Lewis	LE	*FUT3*	6	19p13.3	
008	Duffy	FY	*ACKR1*	5	1q21-q22	CD234
009	Kidd	JK	*SLC14A1*	3	18q11-q12	
010	Diego	DI	*SLC4A1*	22	17q21.31	CD233
011	Yt	YT	*ACHE*	5	7q22	
012	Xg	XG	*XG, MIC2*	2	Xp22.32	CD99†
013	Scianna	SC	*ERMAP*	7	1p34.2	
014	Dombrock	DO	*ART4*	10	12p13-p12	CD297
015	Colton	CO	*AQP1*	4	7p14	
016	Landsteiner-Wiener	LW	*ICAM4*	3	19p13.2	CD242
017	Chido/Rodgers	CH/RG	*C4A, C4B*	9	6p21.3	
018	H	H	*FUT1*	1	19q13.33	CD173

Appendix 2: Blood Group Systems- Part 2 (ISBT, 2018)

No.	System name	System symbol	Gene name(s)*	Number of antigens	Chromosomal location	CD numbers
019	Kx	XK	*XK*	1	Xp21.1	
020	Gerbich	GE	*GYPC*	11	2q14-q21	CD236
021	Cromer	CROM	*CD55*	20	1q32	CD55
022	Knops	KN	*CR1*	9	1q32.2	CD35
023	Indian	IN	*CD44*	6	11p13	CD44
024	Ok	OK	*BSG*	3	19p13.3	CD147
025	Raph	RAPH	*CD151*	1	11p15.5	CD151
026	John Milton Hagen	JMH	*SEMA7A*	6	15q22.3-q23	CD108
027	I	I	*GCNT2*	1	6p24.2	
028	Globoside	GLOB	*B3GALNT1*	2	3q25	
029	Gill	GIL	*AQP3*	1	9p13	
030	Rh-associated glycoprotein	RHAG	*RHAG*	3	6p12.3	CD241
031	FORS	FORS	*GBGT1*	1	9q34.13-q34.3	
032	JR	JR	*ABCG2*	1	4q22.1	CD338
033	LAN	LAN	*ABCB6*	1	2q36	
034	Vel	VEL	*SMIM1*	1	1p36.32	
035	CD59	CD59	*CD59*	1	11p13	CD59
036	Augustine	AUG	*SLC29A1*	4	6p21.1	

Appendix 3: IRB Ethical Approve

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: 12/3/2017
Ref No: 5/REC/2017

Dear Mr. Khalid Younes,

Thank you for submitting your application for research ethics approval. After reviewing your application entitled **"Allo-immonization among transfusion dependent thalassemia patients in the West Bank."** The Research Ethics Committee (REC) confirms that your application is in accordance with the research ethics guidelines at Al-Quds University.

We would appreciate receiving a copy of your final research report/ publication. Thank you again and wish you a productive research that serves the best interests of your subjects.

Dr. Dina M. Bitar
Research Ethics Committee Chair

Cc. Prof. Imad Abu Kishek - President
Cc. Members of the committee
Cc. file

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جامعة القدس
كلية الدراسات العليا
برنامج العلوم الطبية المخبرية
مسار علم الدم
"تحديد الاجسام المضادة لانتيجيات خلايا الدم الحمراء عند مرضى الثلاسيميا في الضفة الغربية"

المشرفون: د. أدهم أبو طه
د. خالد يونس

يعد تكون الاجسام المضادة لانتيجينات خلايا الدم الحرم من أحد اهم الأسباب المرتبطة بمضاعفات نقل الدم ولا سيما عندم المرضى الذين يرتكز علاجهم على نقل الدم بشكل دوري ومنتظم ومن أحد هذه الامراض التي يعتمد علاجها في فلسطين على نقل الدم بشكل دوري منتظم مرض الثلاسيميا.
فانني انا الطالب همام علي طالب في كلية الدراسات العليا في جامعة القدس بصدد اجراء دراسة بحثية للتعرف على الاجسام المضادة لانتيجنات خلايا الدم الحمراء المتكونة عند مرضى الثلاسيميا الذين يعتمد علاجهم على نقل الدم بشكل دوري منتظم بهدف العمل على البحث عن اليات للحد من تكون مثل هذه الاجسام المضادة بهدف التقليل من مضاعفات نقل الدم المرتبطة بوجودها.

ونؤكد لك بأن نتائج هذا الفحص سوف تستخدم لغرض البحث العلمي فقط ويمكنك الاطلاع على نتائج الفحوصات الخاصة بك.

هذا البحث يتضمن بعض الأسئلة عن طبيعة حالتك المرضية وتاريخك المرضي الشخصي والعائلي ومن ثم سيتم سحب عينة دم منك وفحصها لتحديد وجود او عدم وجود الأجسام المناعية المضادة لهذه الانتيجينات.

في حال موافقتك على المشاركة في الدراسة علينا أن نحيطك علما أنه يمكنك الانسحاب من الدراسة في اي مرحلة دون الحاجة لشرح الأسباب ودون التأثير على علاجك وأن معلوماتك ستبقى سرية ولن يتم استخدامها الا لأغراض البحث العلمي.

عملية سحب الدم قد تتضمن خطورة صغيرة لحصول نزف زائد للدم في حال كنت تعاني من أحد امراض نزف الدم او تأخذ أحد المميعات.

في حال كان المريض قاصرا على ولي الأمر ان يوافق على مشاركته ومن ثم التأكد من رأي المريض نفسه.

وشكرا لتعاونكم

توقيع /بصمة المشارك/ولي أمره:

التاريخ:

Appendix 4: Consent Form

Appendix 5: Sample Sheet

Sample number	
Date of collection	
Transfusion centre	
Age	
Gender	
ABO & Rh	
Splenectomy	
Starting transfusion age	
Transfusion Frequency	
Antibody screening	
Antibody identification	

Appendix 6: Characteristics of Alloimmunized Participants

Case Number	ABO	Rh-D	Gender	Age (Years)	Splenectomy	Transfusions Number	Alloantibodies
Case-1	B	Positive	Male	17	Yes	287	Anti-E
Case-2	B	Positive	Female	26	Yes	576	Anti-Kp^a
Case-3	A	Positive	Female	14	NO	208	Anti-E
Case-4	A	Positive	Female	12	NO	572	Anti-E & Anti-K
Case-5	O	Negative	Female	24	NO	564	Anti-D
Case-6	O	Positive	Female	21	Yes	329	Anti-K
Case-7	A	Positive	Male	21	Yes	120	Anti-C
Case-8	AB	Positive	Male	35	Yes	589	Anti-E & Anti-Jk^a
Case-9	A	Negative	Female	22	Yes	372	Anti-C & Anti-D
Case-10	A	Negative	Female	18	Yes	294	Anti-Jk^a
Case-11	AB	Positive	Female	28	Yes	485	Anti-E, Anti-K & Anti- Jk^a

Appendix 7: Participants' demographic

Variable	Alloimmunized	Non-alloimmunized	Total	*P*-value
N (%)	11	90	101	---
Residency				0.089
Ramallah	1	34	35	
Jericho	0	5	5	
Hebron	10	51	61	
Thalassemia Phenotypes				0.321
Major	10	78	88	
Intermedia	1	12	13	
Gender				0.321
Male	3	49	52	
Femal	8	41	49	
Blood Group				0.719
A	5	39	44	
B	2	16	18	
O	2	27	29	
AB	2	8	10	
Status of Rh-D				0.095
Positive	8	81	89	
Negative	3	9	12	
Splenectomy				0.095
Yes	8	40	48	
No	3	50	53	

الكشف عن الاجسام المضادة لمولدات الضد الغريبة عند مرضى فقر دم حوض البحر الابيض المتوسط في الضفة الغربية

اعداد: همام عبد الرحمن حسني علي

اشراف: د. أدهم أبو طه

مشرف ثاني: د. خالد يونس

الملخص

مقدمة:

إن نقل الدم من شأنه أن يقلل بشكل ملحوظ من مضاعفات ومخاطر مرض فقر دم حوض البحر الأبيض المتوسط (الثلاسيميا). لكن مرضى الثلاسيميا بحاجة إلى نقل دم بشكل دوري ومستمر وهذا قد يؤدي إلى تكون أجسام مضادة (Alloantibody) ضد مولدات الضد الغريبة (Foreign Antigens) التي تحملها خلايا الدم الحمراء في الدم المنقول للمرضى.

هذه الأجسام المضادة المتكونة قد تؤدي إلى مضاعفات خطيرة تقلل من درجة استفادة المريض من الدم المنقول وقد تصل المضاعفات إلى درجة قد تشكل خطورة على حياة المريض.

الأهداف:

تهدف هذه الدراسة الى تحديد نسبة تكون الاجسام المضادة (Alloantibody) لدى مرضى الثلاسيميا في جنوب و وسط الضفة الغربية في فلسطين وتحديد انواعها الاكثر انتشارا و تحديد العلاقة بين تكون مثل هذه الاجسام المضادة و عوامل مختلفة كالجنس و العمر عند اول عملية نقل دم و نوع الدم و حالة استئصال الطحال.

طريقة العمل:

تم عمل هذه الدراسة في الفترة الممتدة من شهر شباط من عام 2017 وحتى حزيران من العام نفسه في ثلاث مراكز لمرضى الثلاسيميا في جنوب ووسط الضفة الغربية.

تم جمع 101 عينة حيث تم دراسة المعلومات السريرية والاكلينيكية للمرضى كالعمر ومعدل فترات نقل الدم والعمر عند اول عملية نقل للدم وحالة استئصال الطحال بالإضافة الى نوع الدم. وتم الكشف

عن وجود الاجسام المضادة من عدمه في عينات المرضى المشاركين في الدراسة وايضا تم تحديد انواع هذه الاجسام المضادة.

النتائج

وجد ان 11 عينة من أصل 101 عينة مشاركة في الدراسة تحوي اجسام مضادة (10.9%) اغلبهم كان من الاناث (72.7;8%)، وايضا كان 8 من الذي يحملون اجسام مضادة (72.7%) تم استئصال الطحال لديهم سابقا.

من الذين يحملون اجسام مضادة، 7 (63.6%) كانوا يحملون نوع واحد من الاجسام المضادة، و 4 (36.4%) يحملون نوعين من الاجسام المضادة او اكثر.

تم تحديد 6 انواع مختلفة من الاجسام المضادة ثلاثة منهم تعود لنظام Rh واثنان يعودان لنظام Kell اما النوع الاخير فكان يعود لنظام Kidd.

الخلاصة

اظهرت النتائج ارتفاعا في معدل الاجسام المضادة لدى مرضى الثلاسيميا الذين يعتمدون في علاجهم على نقل الدم. وكانت الاجسام المضادة الاكثر شيوعا تلك التي نتتمي الى نظامي Rh و Kell.

ومن اجل العمل على الحد من مثل هذه الاجسام المضادة فاننا نوصي باتباع سياسات تطابق اوسع في نقل الدم لهؤلاء المرضى ولا سيما تطابق مولدات الاجسام المضادة من نظامي Rh و Kell.