# Alloantibodies amongst Transfusion Dependent Thalassemia and Sickle Cell Disease Patients in the Middle East

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**Abstract:**

The paper provides collective data of numerous studies from the Middle-East regarding alloimmunization rates for both sickle cell and thalassemia patients. A comparison between the alloimmunization rate in the Middle-East and worldwide is made by using representative studies from different countries.

**Abbreviations:**

**RBC = Red Blood Cell**

**SCD = Sickle Cell Disease**

**ISBT = International Society of Blood Transfusion**

**DAT = Direct Antiglobulin Test**

**AHG = Anti-Human Globulin**

**DHTR = Delayed Hemolytic Transfusion Reaction**

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## 1. Background Information:

### 1.1: Blood Group Systems Overview

Blood group system comprises the blood group antigens present on red blood cells that are encoded by certain genes on different alleles or closely linked alleles. The International Society of Blood Transfusion (ISBT) recognizes 33 blood group systems adding to more than 300 blood group antigens (Logdberg, Reid, & Zelinski, 2011). Fivpe blood group systems have carbohydrate structure (ABO, H, P1PK, I, GLOB) and two are found in the plasma (LE, CH/RG) while the rest are proteins integrated within the RBC membrane (Anstee, 2011; Daniels & Reid, 2010).

The most important blood group system is the ABO blood group system. The reason is, humans naturally have antibodies against ABO. The second most important is Rh antigen to which antibodies form only after exposure. However, their clinical importance lies in their antigenicity as well as capability of cross the placenta and causing hemolytic disease of fetus and newborns (Matteocci & Pierelli, 2014).

Sickle cell disease patients and thalassemia who form alloantibodies have antibodies against antigens of the Rh blood groups system, most commonly C and E and to Kell blood group antigens. These findings were consistent amongst different populations (Chou, Liem, & Thompson, 2012)**.** It is important to note than in thalassemia patients, antibodies to the Kell blood group is mostly found in Chinese population rather than in Caucasians (C. K. Cheng, C. K. Lee, & C. K. Lin, 2012).

Other antibodies to the Kidd, Lewis, and MNS blood system antigens are also common. However, antibodies against Lewis blood group is not found to be clinically significant, while for other blood groups they can potentially cause hemolytic transfusion reaction.

### 1.2: Alloimmunization Process

Alloimmunization is a process that happens in the body where a person develops antibodies against foreign antigens (Matteocci & Pierelli, 2014).

Red blood cell alloimmunization is clinically important due to the potential life-threatening hemolytic transfusion reaction. In the past all antibodies that are found in patient sera were considered clinically significant. However, nowadays it is understood that antibodies that react at room temperature are not clinically important (Shulman et al., 1985). Antibodies can affect the survival of transfused red blood cells when they react at 37 C and AHG by different mechanisms such as intravascular/extravascular hemolysis due to activation of complement and interaction of the Fc regions of antibodies with macrophages present in the liver or the spleen (Petz & Garratty,

2004). In multiple transfusion patients such as sickle cell disease patients and thalassemia patients multiple alloantibodies can be found which usually delays the finding of compatible blood as well as expensive laboratory workups (Mangare et al., 2015).

Alloimmunization mostly occurs due to the lack of compatibility between the donor and the recipient. It is mostly seen in cases of racially mismatched blood transfusion in which the recipient and donor are of different ethnicities (E. P. Vichinsky et al., 1990). A compelling evidence for this factor is the reduced prevalence of alloantibody formation in countries where the donor and recipient blood is phenotypically matched i.e. donor and recipient are from the same ethnicity (A. G. Gader, A. K. Al Ghumlas, & A. K. Al-Momen, 2008; B. Natukunda, H. Schonewille, C. Ndugwa, & A. Brand, 2010).

Even in phenotypically matched patients, transfusion dependent patients are still found to form alloantibodies. This is hypothesized to be due to other factors promoting the formation of antibodies. One of these factors is immune status of the recipient. It is indicated that inflammation in the recipient increases the chances of the development of alloantibodies (Hendrickson et al., 2006). In addition to inflammation, dysregulation of the immune system often seen in SCD and B-thalassemia major patients with alloimmunization is another risk factor for the development of alloantibodies in these patients (Bao et al., 2011). To add, splenectomy procedure done for thalassemia patients also puts them at higher risk for the development of alloantibodies (el-Danasoury, Eissa, Abdo, & Elalfy, 2012). To add, a study in the US demonstrated that multi-transfused pediatric patients who were HLA alloimmunized were more likely to develop RBC alloantibodies (McPherson et al., 2010). Other general risk factors are the age of first transfusion, number of blood transfusions, and female gender (Bauer, Wiersum-Osselton, Schipperus, Vandenbroucke, & Briet, 2007; Hendrickson et al., 2006; H. Schonewille, van de Watering, Loomans, & Brand, 2006).

### 1.3: Consequences of Alloantibodies

Alloantibodies in SCD or thalassemia patients can lead delayed hemolytic transfusion reaction (DHTR)upon blood transfusion. The delayed hemolytic transfusion reaction occurs by antibodies present in the recipient blood attacking the transfused RBCs and hemolyzing them. Sometimes, these alloantibodies might not be detected at the time of DHTR, could be detected later or not at all (Garratty, 1997). The hemolysis occurring leads to worsening of the patient condition and increasing in the anemic state of the patient due to hemolysis, hyper-hemolysis, and suppression of erythropoiesis (Rosse, Narla, Petz, & Steinberg, 2000). Sometimes, a phenomenon called bystander hemolysis occurs. In which, cells that are negative for the antigen the antibody is working against will undergo hemolysis due to complement activation (Garratty, 1997; King, Shirey, Lankiewicz, Young-Ramsaran, & Ness, 1997).

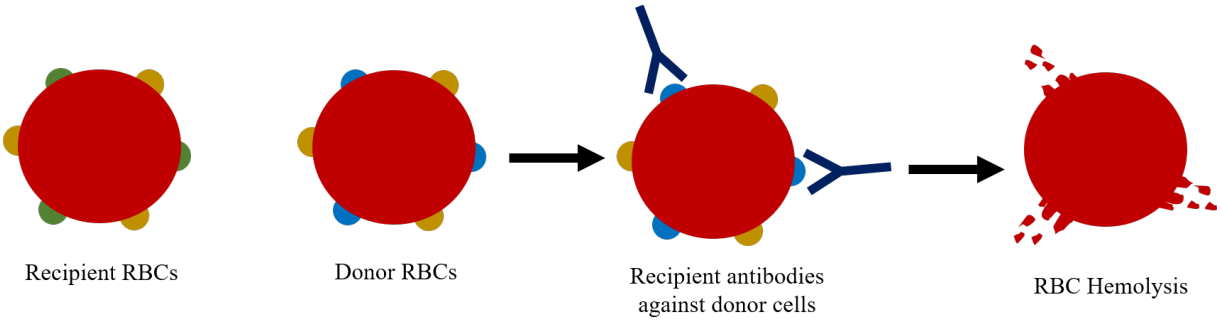


**Diagram 1:** When the donor has antigens that the recipient doesn't have, there is a high possibility that the recipient develops antibodies against these antigens. These antibodies eventually lead to the hemolysis of red blood cells causing delayed hemolytic transfusion reaction.

### 1.4: Formation of Autoantibodies

Autoantibodies are frequently seen in patients with sickle cell anemia as well as thalassemia who are chronically transfused. The presence of autoantibodies is indicated by positive DAT. Of the risk factors for the development of autoantibodies are existing alloantibodies, inflammation, as well as gene polymorphism (E. Vichinsky, 2012). There is a convincing evidence linking the formation of alloantibodies in transfusion dependent patients with the formation of autoantibodies. It is typical to find patients with alloantibody formation to form non-specific warm autoantibodies (Lasalle-Williams et al., 2011)**.** The formation of autoantibodies can occur simultaneously with the formation of alloantibodies, or it can be preceded by the alloantibody formation (Venkateswaran, Teruya, Bustillos, Mahoney, & Mueller, 2011)**.** It is important to know that sickle cell disease patients also have a predisposition to develop autoantibodies to specific antigens other than red blood cell antigens which are thought to be due to immune related dysfunction (Toly-Ndour et al., 2011)**.** It is thought that autoantibodies formed in sickle cell disease patients are independent of blood transfusions and can be also found in patients who were not transfused (Kangiwa, Ibegbulam, Ocheni, Madu, & Mohammed, 2015). On the other hand, autoantibodies formed in thalassemia patients is highly associated with the presence of alloantibodies due to multiple transfusion reactions (Kruatrachue, Sirisinha, Pacharee, Chandarayingyong, & Wasi, 1981). To presence of autoantibodies in the thalassemic patients blood lead to acceleration of hemolysis by reducing the life span of red blood cells (Ameen et al., 2003). Moreover, alloantibodies and autoantibodies in thalassemic patients are often associated with marked lymphocytosis, specially in B-lymphocytes. This is also accompanied by an increased level of immunoglobulin directed against red blood cell antigens. This is especially seen in patients who undergone splenectomy (S. T. Singer et al., 2000).

When auto-antibodies are found, identification of those antibodies which includes their type and the temperature they work at. Also, elution of autoantibodies is necessary to detect alloantibodies, if any are present. More importantly, positive DAT could be due to sensitization of donor RBCs after transfusion, so transfusion history is important to be checked. Another

crucial factor is to check if the patient is taking any medications which could yield a positive DAT such as cephalosporins (E. Vichinsky, 2012).

### 1.5: Thalassemia

Thalassemia is known as one of the common genetic disease affecting the world's population. Worldwide between 100,000 and 200,000 persons are born with severe forms of the disease. The prevalence of thalassemia syndromes is high in Mediterranean basin (hence the name; thalassa which means sea in Greek), Middle East, tropical Africa, and Asian (Muncie & Campbell, 2009). Thalassemia is an inherited disease where one or more genes of the globin of hemoglobin is mutated which lead to either reduction or absence of corresponding globin chain (McKenzie, Williams, & Landis-Piwowar, 2015). The severity of the disease depends of the type of chain being affected, and the number of the chain produced. Since six globin genes exist (α, β, δ, ε, γ, ζ), six or more versions of thalassemia are possible. People inheriting this disorder could be asymptomatic when the genetic defect is mild, or they could be symptomatic when genetic defect is severe. The symptoms observed in patient with the severe form of disease include anemia, hepatosplenomegaly, gallstone, and infections which result from either low production level of hemoglobin, abnormal hemoglobin formation, imbalanced synthesis between globin chains which could affect erythropoiesis, or all of them combined (Kumar, Abbas, Aster, & Robbins, 2015).

Thalassemia is classified to different groups depending on the globin chain affected. The most clinically significant type of thalassemia is the one affecting the alpha and beta chains since they are component of hemoglobin A (major type of normal adult hemoglobin). α-thalassemia and β thalassemia results from either reduction or absence of α globin chain or β globin chain respectively (Melody J Cunningham, 2008).

#### 1.5.1: α-Thalassemia

Is characterized by decrease in the synthesis of α chains. Mutations could affect one or more of α genes that results in four distinct clinical manifestations. The amount of α chain produced in proportional to the number of alleles affected (not always the case). α thalassemia founds mainly in Mediterranean, African ancestry (because the deletion involves $\alpha_1$ gene in these ethnic population), and Asian (Higgs & Weatherall, 1983).

*Hydrops fetails* is a condition where all four α genes are deleted therefore no production of α chains. *Hemoglobin H* disease is a condition where three of the four α genes are deleted. Deletion of two α genes is known as *α thalassemia minor*, while deletion of only one α gene is known as *silent carrier*. Although nondeletional mutations and mutations that lead to the formation of unstable α chains are reported, they are rare (Komvilaisak et al., 2017).

The most severe form of α thalassemia is α thalassemia major where all four α genes are deleted. As no production of α genes occur, no healthy and useful hemoglobin could be produced, therefore, infants are either die within hours of birth or they stillborn. Some physical abnormalities are seen on infants who survive until birth. These babies have enlarged liver and spleen (extramedullary hematopoiesis) and they are born underweight. Massive bone marrow hyperplasia, hemolysis and extensive deposition of hemosiderin are also seen in these babies (Embury et al., 1980; Kan, Schwartz, & Nathan, 1969).

Laboratory diagnosis show anemia with hemoglobin level ranging from 4-10 g/dL. Red blood cells are microcytic and hypochromic. HbA, $HbA_2$, and HbF are present since no α chain is produced.

α *thalassemia minor*/ trait occurs when two out of the four α genes are deleted either on the same of opposite chromosomes. The condition is observed in all geographic locations. Since not all the α genes are deleted, the unaffected α globin genes are able to compensate for the reduction of α globin synthesis.

**1.5.2: β Thalassemia**

β thalassemia is occur as a result of different types of molecular defects. Although two hundred mutations result in either partial or complete absence of β gene expressions, only 20 mutations account for 80% of diagnosed β thalassemia. The form of the disease ranges from minor reduction of the β globin reduction to absence of the globin synthesis. Different steps could be affected due to these mutations such as gene transcription, mRNA translation, RNA processing, and post- translational integrity of the protein. Four forms of the disease are seen (β thalassemia major, β thalassemia intermedia, β thalassemia minor, and β thalassemia minima), and the classification of these conditions depend on severity of symptoms, medical interventions, and prognosis. The most severe mutation is ($\beta^0$) and it is frequently found in the

Mediterranean regions (Italy, Greece, Algeria, Saudi Arabia, and Southern Asia) (Rund & Rachmilewitz, 2005).

β thalassemia major known also as *Cooley's anemia* caused by either homozygous or double heterozygous inheritance of abnormal β genes which could cause reduction or absence of β chain synthesis. The pathologic consequences behind this reduction of absence of β chain are; reduced HbA which affect the blood's oxygen carrying capacity, compensatory production of other hemoglobin, hemolysis occur mostly at the polychromatophilic erythroblast stage, ineffective erythropoiesis, and erythroid hyperplasia (M. J. Cunningham, Macklin, Neufeld, Cohen, & Thalassemia Clinical Research, 2004). Hemoglobin level could be as low as 2 or 3 g/dl, and red blood cells morphology will be microcytic and hypochromic. The peripheral smear will be composed of anisocytosis and poikilocytosis. Nucleated erythrocytes are observed while the RDW could be either normal or increases. If bone marrow performed (not necessary) erythroid hyperplasia is observed with an M: E ratio of 1:10 or less. Children with β thalassemia major undergo regular transfusion program which helps in prolonging the survival rate of the child until the second or third decade (Galanello & Origa, 2010).

β thalassemia minor occur due to heterozygous inheritance of either a $\beta^{+}$ or $\beta^{0}$ gene while the other β gene is normal. In this form of the disease, the normal β gene directs the synthesis of sufficient amount β chain that will help in synthesizing enough HbA for normal erythrocyte survival and oxygen delivery. Patients with β thalassemia minor appears to be asymptomatic most of the time except during infections and pregnancy. Moderate microcytic anemia could be seen. In case of folate deficiency macrocytic anemia could develop. The hemoglobin level values are in the range of 9-14 g/dl. Red blood cells are microcytic and hypochromic (could be normochromic). Variable anisocytosis and poikilocytosis could be seen. Nucleated red blood cells are not usually observed. Bone marrow testing shows slight erythroid hyperplasia. Patients with β thalassemia minor generally do not require treatment if they retain good health and nutrition (Sheiner, Levy, Yerushalmi, & Katz, 2004).

β thalassemia intermedia occur due to the inheritance of homozygous, double heterozygous, and heterozygous. β thalassemia intermedia present with intermediate symptoms between the severity of β thalassemia major and mild β thalassemia minor. Moderate microcytic hypochromic anemia with hemoglobin value ranges between 7-10 g/dl is seen. Target cells are

predominant poikilocytosis, basophilic stippling, and nucleated red blood cells are observed. Although bone marrow examination is not needed for the diagnosis of the disease, the result shows hypochromic erythroblast picture (Ben Salah, Bou-Fakhredin, Mellouli, & Taher, 2017).

β thalassemia minima is an asymptomatic form of β thalassemia where no major laboratory abnormalities are seen. It is defined as mildly imbalanced α to non α globin chain synthesis ratio. The genotype used to describe a patient with β thalassemia minima is $\beta^{SC}$/ β. Homozygosity of $\beta^{SC}$ or combination of silent allele and $\beta^{-}$ thal genes result in mild form of β thalassemia intermedia (McKenzie et al., 2015).

### 1.6: Sickle Cell Anemia

Sickle cell anemia is considered the most common hemoglobinopathy worldwide with a higher prevalence in Tropical Africa(Bender & Seibel, 2014). Sickle cell gene is also prevalent in the Mediterranean, India, Nepal, and the Middle East (Kondani et al., 2014). Statistic obtained from African Americans participants showed that sickle cell anemia occurs in 0.3-1.3%, while sickle cell trait occurs in 8-10% (Bae et al., 2012).

#### 1.6.1: Pathophysiology

Sickle cell anemia is caused by a mutation in the *HBB* genes which lead to a conversion in the amino acid from GAC to GTG. This mutation lead to the production of nonpolar valine instead of polar glutamic acid at the sixth amino acid position, which lead to the formation of mutant hemoglobin HbS. HbS solubility in the deoxygenated state is reduced significantly that lead to rigid aggregation of deoxyhemoglobin S molecules (Strouse, 2016). Depending on the polymerization extend of HbS, the morphology of red blood cells could have the crescent shape. The HbS polymer formation depends on many factors such as hypoxia, acidosis, hypertonicity, and temperature when its below 37 °C (Health, 2009). Although reoxygenation of the hemoglobin could return sickled erythrocytes to their normal morphology (biconcave shape), red blood cells with repeated sickling episodes could become irreversible sickled cells where the shape of cells stays sickled whether deoxygenated or oxygenated (Shatat et al., 2013).

### 1.6.2: Clinical Findings

The appearance of the clinical signs of sickle cell anemia appears during the first six months of age where the HbS concentration predominates HbF. A moderate to severe form of anemia could be seen due to extravascular hemolysis. The high level of bilirubin turnover and cholestasis could lead to the development of gallstones. Folate deficiency is also could be seen which lead to megaloblastosis (Kumar et al., 2015).

### 1.6.3: Laboratory diagnosis

The characteristic of sickle cells anemia peripheral blood is classified as normocytic and normochromic anemia with marked reticulocytosis. The hemoglobin level ranges from 6-10g/dl, while the hematocrit ranges from 18-30%. The blood smear shows alterable anisocytosis, variable poikilocytosis, sickled cells, and target cells. Splenic hypofunction could be observed in older children and adults. Different procedures could be obtained to diagnose sickle cell anemia such as; bone marrow aspiration, hemoglobin electrophoresis, solubility tests, and sickling test (McKenzie et al., 2015).

### 1.6.4: Treatment

Immediate transfusion therapy could be used to reduce the complication of sickle cell disease. Preoperative transfusion is also helpful that prevent the complication of anesthesia-induced sickling. Some pharmacologic agents could be used which help reducing the sickling by increasing the level of HbF (ex: hydroxyurea (HU)) (De, 2008).

## 2. Alloimmunization in the Middle-East and Around the World:

### 2.1: Alloimmunization rates for SCD and thalassemia in the Middle-East

Data on alloimmunization in that middle east is not sparse. However, there were some studies that were published calculating the frequencies of alloimmunization and the most frequently seen alloantibodies.

Two studies from Sudan calculated the frequency of alloimmunization on patients with sickle cell disease. The first study was carried out by (Abbas, Bolad, Jiefri, & Mergani, 2013) in Omdurman having 100 patients of age between 6 months to 17 years. Out of 100 patients, only 4 developed alloantibodies (4%) and the antibodies formed were against Kell, E, and C antigens. It is important to note that 45% of the patients in this study were transfused from relatives. 50% of the alloantibodies were anti-K, while 25% were anti-E and 25% anti-C (Abbas et al., 2013).

Lower alloantibody formation rate in this study could be due to 45% transfusion from relatives as well as early age of the study group. The second study included children with sickle cell disease in Elobeid Teaching Hospital. The study population consisted of 210 patients (Eldour, Ismail, Osman, & Babker, 2015). The rate of alloantibody formation in this study is 4.3% of that, 50% had anti-K and 20% developed anti-E (Eldour et al., 2015). It is important to note that the results in this study show low alloimmunization rate amongst children and it is not possible to deduce the rate of alloimmunization of sickle cell disease patients in Sudan from these studies as they didn't include adult population of Sudan. To add, a study in Egypt done by (Aly, El-sharnoby, & Hagag, 2012) indicates 42 patients with sickle cell disease who have undergone multiple transfusion were tested for the presence of alloantibodies. 21.4% of the patients developed alloantibodies with the most common antibodies anti-K followed by anti-E and anti-C.

From the gulf region a Kuwaiti study published by (Ameen, Al Shemmari, & Al-Bashir, 2009) showed that amongst 233 Kuwaiti Arab sickle cell disease patients, 43.3% developed clinically significant antibodies. It is important to note that in this study, the study population was divided into two groups, one that received leuko-reduced blood and the other didn't. The results of the study showed that the group receiving non-leuko-reduced blood had a significantly higher alloimmunization rate than the group receiving leuko-reduced blood in rates 65.5% and 23.6% respectively. (Ameen et al., 2009) also indicated that most of the alloantibodies formed were against antigens in the Rh and Kell blood group system. Another study from Saudi Arabia by (Adam & Badawi, 2017) showed 17.8% rate of alloimmunization amongst sickle cell disease patients. With the most common type of antibodies found to be anti-E (18.8%) and anti-K (12.5%). This study also showed that older age and female gender contributed to alloimmunization.

In addition, some studies studied alloimmunization in both B-thalassemia patients and sickle cell disease patients and combined their results. These studies will be discussed below. The first one is done in Oman by (Alkindi et al., 2017) which indicated that 20% of thalassemic patients developed alloantibodies. Like other studies the most common antibodies were anti-K and anti-E with frequency of 26.9% and 23.1% respectively. In SCD patients 31.6% of the patients developed alloantibodies. (Alkindi et al., 2017) suggests that these high alloimmunization rates are due to the Omani population being a mixture of different ethnic groups. Concerning the same study, the rate of alloimmunization in SCD patients is higher than that of thalassemia patients which could due to thalassemia patients being transfused at younger age and with more consistency than sickle cell disease patients. Also, since SCD leaves individuals affected with it at a chronic inflammatory state, this state promotes the formation of alloantibodies.

Moreover, a study carried out by (Al-Mousawi, Al-Allawi, & Alnaqshabandi, 2015) provided the first published resource for data on alloimmunization in Iraq. The study was done in a center in Kurdistan. The study included 311 patients with B-thalassemia and 90 patients with sickle cell disease. All patients received leuko-reduced blood. Alloantibodies formed in only 4.5% of patients. The alloantibodies formed were: anti-E, anti-D, anti-K, and anti-$C^{w}$. There was significant increase in alloimmunization in patients receiving their first blood transfusion after 2

years of age. Ethnicity also showed significance in relation to development of alloantibodies. In which, Kurdish Muslims (which comprise majority of the population of Kurdistan) had lower rates of alloimmunization. This could be attributed to the fact that the blood is ethnically matched. It is important to know that even though anti-D alloantibodies were the most frequent, the blood given to the patients was ABO and RhD matched. This could be due to the failure of detection of RhD variants in donors (Al-Mousawi et al., 2015).

Another study in Saudi Arabia studied the prevalence and frequency of alloimmunization in both sickle cell disease patients and thalassemia patients. According to (A. G. M. A. Gader, A. K. Al Ghumlas, & A. K. M. Al-Momen, 2008) the frequency of alloantibody formation in 68 patients in this study was 22.06%. However, of these patients, the ones receiving racially matched blood (19.12%) didn't develop any antibodies, while the ones receiving non-racially matched blood which comprised 69% of the total test population had 21.3% rate of alloantibody formation.

The rest of the studies were focused on B-thalassemia patients and are of different geographical areas in the middle east: Turkey, Iran, and Egypt. Two different studies from Egypt showed considerably variable results. The first study by (El Sewefy et al., 2014) done on pediatric thalassemia patients age less than 16 years old at Ain Al-Shams University Hospital. The study found that 10.5% of patients developed alloantibodies and majority of them were anti-Kell (52.4%) and anti-E (19%). Moreover, (Saifeldeen, Awad, El-Tonbary, Aladle, & Elghannam, 2017) indicated out of 65 patients 23% of the cases had alloantibodies. Only 6.2% of patients had both alloantibodies and autoantibodies. Majority of antibodies were against Rh (6.2%) and Kell (12.3%). The also study found significance between age of first transfusion and formation of alloantibodies.

A cross sectional study by (Koçyiğit, Eliaçık, Kanık, Atabay, & Türker, 2014) done on patients with thalassemia who received regular blood transfusion of leukodepleted RBCs. The study group comprised 139 patients of different age groups suffering from B-thalassemia intermedia and major who were regularly transfused. Only 6.4% of patients had alloantibodies in their blood while 12.2% had autoantibodies. Autoantibody development rate was higher in people who undergone splenectomy and older people. The antibodies formed were mostly anti-K which had a frequency of 27% and anti-C with a frequency of 27% (Koçyiğit et al., 2014). It is interesting to note that anti-E had a comparatively low frequency which was 9%. This is unlike other studies discussed in this paper where anti-K and anti-E resemble majority of antibodies formed.

Further, an Iranian study was done in Zanjan province in Iran on B-thalassemia patients (Davari & Soltanpour, 2016). 49 patients were tested and the percentage of patients developing alloantibodies was 16.32%. The antibodies that were found were 60% anti-Kell , and 10% of anti-c, anti-E, and anti-$Le^b$ each. Patients who received leuko-reduced blood had significantly less antibody formation. In this study gender was found to be insignificant to alloantibody formation (Davari & Soltanpour, 2016). Likewise, a meta-analysis by (Darvishi et al., 2016) including 297 studies from different cities in Iran. The study concluded that the prevalence of alloimmunization in B-thalassemia patients in Iran is around 10% . Of this 10%, most common is anti-K (37%), anti-D (29%), and anti-E (20%). The writers note that the immunization with anti-

D is very high although Rh D pretransfusion testing in mandatory in Iran. Which is thought to be a result of the presence of undetected D-variants in the Iranian population (Darvishi et al., 2016).

**Table 1: The table shows data collected from different studies in the Middle East in which the frequency of alloimmunization and most common antibodies are illustrated for sickle cell disease and thalassemia patients. It can be deduced from this table that the frequency of alloantibodies formation varies widely from one country to another, even within countries, to give a range between 4.0% - 43.3%.**

| First Author | Country | Disease | Frequency of Alloimmunization | Most Common Antibodies |
|---|---|---|---|---|
| (Abbas et al., 2013) | Sudan | Sickle Cell | 4.0% | 50.0% anti-K<br>25.0% anti-E<br>25.0% anti-C |
| . (Eldour et al., 2015) | Sudan | Sickle Cell | 4.3% | 50.0% anti-K<br>20.0% anti-E |
| (Aly et al., 2012) | Egypt | Sickle Cell | 21.4% | 33.3% anti-K<br>22.2% anti-E<br>22.2% anti-C |
| (Ameen et al., 2009) | Kuwait | Sickle Cell | 43.3% | N/A |
| (Adam & Badawi, 2017) | Saudi Arabia | Sickle Cell | 17.8% | 18.8% anti-E<br>12.5% anti-K |
| (Alkindi et al., 2017) | Oman | Sickle Cell &<br>Thalassemia | 31.6%<br>20.0% | 26.9% anti-K<br>23.1% anti-E |
| . (Al-Mousawi et al., 2015) | Iraq | Sickle Cell &<br>Thalassemia | 4.5% | Anti-E<br>Anti-D |
| . (A. G. M. A. Gader et al., 2008) | Saudi Arabia | Sickle Cell &<br>Thalassemia | 22.1% | 33.3% anti-K<br>22.2% anti-E |
| . (El Sewefy et al., 2014) | Egypt | Thalassemia | 10.5% | 52.4% anti-K<br>19.0% anti-E |
| (Saifeldeen et al., 2017) | Egypt | Thalassemia | 23.0% | 26.6% anti-K<br>13.3% anti-k<br>13.3% anti-D |
| (Koçyiğit et al., 2014) | Turkey | Thalassemia | 6.4% | 27.0% anti-K<br>27.0% anti-C |
| (Davari & Soltanpour, 2016) | Iran | Thalassemia | 16.3% | 60.0% anti-K<br>10.0% anti-c<br>10.0% anti-E<br>10.0% anti-$Le^b$ |
| (Darvishi et al., 2016) | Iran | Thalassemia | 10.0% | 37.0% anti-K<br>29.0% anti-D<br>20% anti-E |

## 2.2: Prevalence of alloantibodies in sickle cell patients worldwide

Development alloantibodies is a well-recognized phenomenon in sickle cell anemia and thalassemia patients. Nevertheless, there are no established statistics on the prevalence of alloimmunization worldwide. Since we have provided in an earlier section the prevalence of alloantibodies in the middle east with the most common antibodies formed, it is logical examine the distribution of this phenomenon worldwide.

In Europe, a Dutch paper examined the frequency of alloimmunization among 245 SCD patients and revealed that 18% of these had alloantibodies. Half of these patents developed alloantibodies before they received their $8^{th}$ unit. This study took into consideration the introduction of extended matching for RhCE and Kell antigens. Alloimmunization was compared between patients who received extended matched units and those who did not. It was concluded that those who received non-extended matched units were at higher risk to develop alloantibodies (Sins et al., 2016).

In the African content 365 Tanzanian SCD patients were tested for the presence of alloantibodies. 4.1% of these patients tested positive for alloantibodies. Majority of alloantibodies belonged to the kell system (26.2%), Rh system (18%), and Lewis system (18%), MNS system (9.8%) and Colton system (8.2%). More than half of the patients had multiple antibodies(Meda et al., 2014). In neighboring Uganda, a study on 428 SCD patients demonstrated that 6.1% of these patients developed alloantibodies. Majority of alloantibodies belonged to the Rh system (66.7%) and MNS system (16.6%)(Bernard Natukunda, Henk Schonewille, Christopher Ndugwa, & Anneke Brand, 2010).

Similar to the case of alloimmunization in adults, the available literature does not contain enough information on the prevalence of alloimmunization in pediatrics with hemoglobinopathies and multiple transfusions patients. A French study on 175 SCD pediatric patients demonstrated a 7.4% prevalence of alloimmunization amoung these children. The study also suggest that increase in number of RBC units received and presence of preexisting autoantibodies increases risk of alloimmunization. (Allali et al., 2017).

It Is worth to mention that the CDC funded a study on 90 patients to determine the effect of minor antigens and alloimmunization on patients with SCD. The patients were given matched blood for the most common antigens. The study concluded that the presence of preexisting alloantibodies increased the risk for the development of more alloantibodies(Yee et al., 2017).

## 2.3: Prevalence of alloantibodies in thalassemic patients worldwide

Starting with the country harboring the largest population in the world, 382 Chinese patients suffering from thalassemia major were tested for alloantibodies. Of these patients 18.3% developed alloantibodies and the majority had single antibodies detected. Anti-E (39.3%), anti-Mia/Mur (30.85%), and anti-c (13.1%) were the most common detected alloantibodies. Only one patient developed anti-K and 2 developed anti-Fyb antibodies(C. Cheng, C. Lee, & C. Lin, 2012). Moving to the country with the second largest population, 319 multi-transfused Indian thalassemia patients were tested for alloantibodies. 5.64% of these patients tested positive for the

presence of alloantibodies, majority of which were directed against Rh system (52.17%) followed by Kell (35%), Kidd (9%) and Xg (4%) blood systems. This study also found that patients with thalassemia intermedia were more likely to develop alloantibodies(Dhawan et al., 2014). In Eastern India 500 multi-transfused thalassemia patients were screened for alloantibodies. A prevalence of 5.6% of alloimmunization was determined. 52.17% of total alloantibodies found belonged to the Rh system followed by alloantibodies against antigens form the Kidd system(Datta, Mukherjee, Talukder, Bhattacharya, & Mukherjee, 2015).

The Mediterranean is known for its high prevalence of thalassemia patients. A study was carried on 118 Albanian thalassemia patients who were transfused with ABO, Rh and Kell matched blood units for five years. 10.1% of these patients had alloantibodies before the beginning of the study. Most common alloantibodies belonged to the Rh system followed by the Kell blood group. After five years, only 1.7% patients developed alloantibodies (one anti-Cw and one anti-Jkb). The outcome of this study supported evidence that giving extended matched RBC units greatly reduces the risk of the development of alloantibodies (Seferi et al., 2015).

The high prevalence of thalassemia in the African continent emphasis the need for more data to be collected on the incidence of alloimmunization among African patients. In a study carried on 130 Tunisian thalassemia patients to determine the alloimmunization rate, 7.7% of the patients were found to have alloantibodies. Most of these alloantibodies were directed against Rh system followed by anti-Kell and anti-S (Guirat-Dhouib et al., 2011).

697 American thalassemia patients undergoing transfusion therapy were tested for alloantibody production. 16.5% of the patients developed alloantibodies, most of which were directed against Rh antigens follows by anti-Kidd, anti-Kell, anti-Lewis, anti-MNS and anti-Duffy. Half of the patients had multiple alloantibodies. After being age adjusted, alloimmunization rate was not affected by race or gender(Thompson et al., 2011). 64 patients with thalassemia were tested for alloantibodies. 48 (75%) of these patients were from an Asian descendant. 14 (22%) of the patients developed autoantibodies, from which 10 (15.6%) were Asian descendants. In the Asian population 4.8% of the patients developed alloantibodies. Although these patients are residents of the US, they are of Asian descendant, which indicates that they have different phenotypes. Most of the blood donors are whites, and this lead to the increased rate of alloimmunization amoung Asian descendants (Sylvia T Singer et al., 2000). Generally, the alloimmunization in the United States is considerably more prevalent than other areas of the world (except China). This could be attributed to the fact that the United States has a more diverse population compared to other areas of the world. This results in the reduction of the likelihood of random matching between donors and recipient beyond ABO and Rh.

**Table 2: This table shows collective data from studies in different countries around the world. The frequency of alloimmunization and the common antibodies formed are illustrated with respect to the disease (sickle cell anemia or thalassemia). Wide variation between different countries across the world can be seen both in alloimmunization rate and the most common antibodies formed. The range of alloimmunization frequency is between 4.1% and 20.8% with the lowest rate in Tanzania and the highest in Asians living in the United States.**

| First Author | Country | Disease | Frequency of alloimmunization | Most common antibodies |
|---|---|---|---|---|
| . (Bernard Natukunda et al., 2010) | Uganda | Sickle Cell Anemia | 6.1% | 33.3% anti-E<br>23.3% anti-D<br>13.3% anti-S |
| (Meda et al., 2014) | Tanzania | Sickle Cell Anemia | 4.1% | 19.6% anti-K<br>14.8% anti-$Le^a$<br>8.2% anti-Cob |
| (C. Cheng et al., 2012) | China | Thalassemia | 18.3% | 39.3% anti-E<br>30.85% anti-Mia/Mur<br>13.1% anti-c |
| (Datta et al., 2015) | Eastern India | Thalassemia | 5.6% | 28.6% anti-c<br>21.4% anti-E<br>7.1% anti-$Jk^b$ |
| (Dhawan et al., 2014) | India | Thalassemia | 5.64% | 35.0% anti-K<br>17.0% anti-E<br>13.0% anti-D<br>13.0% anti-C |
| (Seferi et al., 2015) | Albania | Thalassemia | 11.8% | 23.9% anti-K<br>19.1% anti-D<br>14.3% anti-C<br>14.3% anti-E |
| (Sylvia T Singer et al., 2000) | USA (Asians) | Thalassemia | 20.8% | 8.3% anti-K<br>2.1% anti-E<br>2.1% anti-c |
| (Thompson et al., 2011) | USA | Thalassemia | 16.5% | 19.0% anti-E<br>18.1% anti-K<br>9.5% anti-C |
| (Guirat-Dhouib et al., 2011) | Tunisia | Thalassemia | 7.7% | 40.0% anti-E<br>40.0% anti-C<br>20.0% anti-D<br>10.0% anti-K |

### 2.4: Comparison Between Studies From the Middle East and the World

In previous sections the alloimmunization rates and data from selective studies in the Middle East and different countries around the world were described. It was noted that the Middle-Eastern studies have shown alloimmunization rates between 4.0% to 43.0% while worldwide studies from different countries have shown lower range which was between 4.1% and 20.8%. The highest rate of alloimmunization noted previously is found in Kuwait in sickle cell disease patients. According to (Ameen et al., 2009) , the study used two groups of patients, one group received non-leuko-reduced blood that is only ABO and Rh-D matched while the other group received. However, for the second group Rh antigens matching and Kell antigen matching was done as well. The total alloantibody formation in these two population was found to be 43%. Therefore, the high percentage could be due to the use of non-leukoreduced blood that is only ABO and Rh-D matched. It can be seen that homogenous populations in the Middle-East as well as worldwide show lower rates of alloimmunization on general in comparison to populations of individuals of different ethnicities. This can be attributed to antigenic variations amongst these ethnicities.

To add, the most common antibodies formed in Middle Eastern patients were anti-K followed by anti-E in most studies. There were exceptions in the study by (Saifeldeen et al., 2017) , where anti-K, anti-k and anti-D were most prevalent. In contrast to Middle Eastern studies, worldwide studies have shown a lot of variation with anti-E antibody showing in all of the studies while anti-K showing in majority but not all. Uncommon antibodies were formed in certain populations were also found. For example in a chinese study by (C. Cheng et al., 2012) , anti-Mia/Mur antibodies were found to be prevalent in 30.85% of thalassemic multiramified patient who were alloimmunized. This is possibly due to the high prevalence of these antigens in South-East Asia especially in China and Taiwan (Prathiba, Lopez, & Usin, 2002).

## 3. Prevention of Alloantibody formation:

The cause behind the formation of alloantibodies in sickle cell anemia and thalassemia patients is the exposure to red blood cell antigens that are foreign to their own during blood transfusion process.

Extending RBC phenotyping in which all the antigens present on the recipient RBCs are typed, Genotypic studies can also be done (Allen et al., 2009). The importance of genotypic studies is an example of altered C antigens which are found in up to 20% of African Americans (Tournamille et al., 2010). Patients with this altered antigen type C+ but also make antibodies against C antigen (Tournamille et al., 2010). Unfortunately, there is no consensus agreeing on benefits outweighing the costs of extended phenotyping, thus it is not done in many centers unless alloantibody has already formed (Afenyi-Annan & Bandarenko, 2006). From the studies in the middle east that were reviewed in the previous section. It was found that most centers in the middle east only use ABO and Rh-D matched red blood cells.

Using leukocyte reduced blood is a standard procedure in many centers transfusing patients with sickle cell disease or thalassemia (Spinella et al., 2010). The effect of using leuko-reduced blood varies from one study to another. Some studies show that using leuko-reduced blood significantly reduces alloantibody formation while others show that it has no effect (Davari & Soltanpour, 2016; Henk Schonewille & Brand, 2005; Wang et al., 2006). The goal of leukoreduction is to reduce the risk of having a febrile reaction and the transmission of cytomegalovirus. That is, febrile reactions could lead to the ending the transfusion thus, leading to failure of achieving goal hemoglobin or hematocrit (Blajchman, 2006).

Enhancing donor recruitment especially in populations where ethnicity is not homogenous. One suggestion by () is to more frequently recruit people from African descent who usually lack $Fy^a$, $Fy^b$ , C, E, K, S, and $Jk^b$ antigens. This has been done in the US to transfuse patients with sickle cell disease especially (Shaz, James, Demmons, Schreiber, & Hillyer, 2010). Also, rare donor programs could be established to provide RBCs lacking commonly encountered antigens. Another strategy to reduce the risk of alloimmunization is to use a limited number of donors for the same patient, or even the same donor (Hillyer, Blumberg, Glynn, & Ness, 2008). Finding dedicated donors for multiple-transfusion patients is an effective strategy to reduce the rate of alloimmunization (El Danasoury, Eissa, Abdo, & Elalfy, 2012; Roberts et al., 2012).

**4. Conclusion:**

Alloimmunization is a common consequence of blood transfusion in patients who receive multiple blood transfusion especially sickle cell disease patients and thalassemia sufferers. The rates of alloimmunization around the world and in the Middle East vary due to the variability in ethnic homogeneity in those countries as well as the preventative measures in each country such as providing leukoreduced blood or the availability of Rh and kell antigens matching.

The most common antibodies found in the middle east due to alloimmunization are against antigens in the kell system as well as Rh. Thus, matching for these blood group systems with the addition of using leukoreduced blood would act as a preventative measure for most alloimmunization reactions.

Using data from published studies in different countries worldwide showed that the rates of alloimmunization in the Middle-East are comparable to the ones present worldwide. However, the type of antibodies formed differ between countries due to the difference in population genotypes and phenotypes.

Finally, seeking prevention of alloimmunization amongst multiple transfusion patients is important in order to avoid future complications in finding matching red blood cells which could compromise the patient life if no matching red blood cells were found.

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