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Detection of Alloimmunization Rate with Anti-D in Rh/D-Negative Patients with Diverse Immune Status After Receiving Rh/D-Positive Red Blood Cells

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ABSTRACT

Commuting Rh/D-negative patients to receive Rh/D-positive red blood cells are necessary in emergency circumstances. The rate of anti-D alloimmunization after administering Rh/D- positive red blood cells to Rh/D-negative patients is affected by age, gender, immune status, the number of Rh/D-positive red blood cells units transfused and the duration of antibody formation. Controlling and understanding the effects of these variables on increasing the chance of alloimmunization allow treating physicians to practice switching Rh/D- negative patients to receive Rh/D-positive red blood cells more safely. Different statistical approaches were implementedto retrospectively analyse of cross-sectional observation on 106 Rh/D-negative patients 50 patients were immune competent while 56 patients were immune compromised who received Rh/D-positive red blood cells transfusion. Descriptive statistics was used to describe variables affecting rate of anti-D alloimmunization. Bivariate analysis and probable associations were used to evaluate differences between factors affecting alloimmunization rate among patients. Maltivarate analyses was used to determine factors affecting alloimmunization rate. 6 out of 106 (5.6%) Rh/D-negative patients received Rh/D-positive red blood cells were alloimunzed with different blood group Antigens (NSW, Jk^b and E). 4 of 6 alloimunzed patients with Anti Rh/D were immune competent patients. Alloimmunization was lower in immune compromised patients only 2 out of 6 alloimunzed patients. This study is the first in the Saudi Arabia that determine the rate alloimmunization in Rh/D-negative patients with diverse immune status, who were exposed to Rh/D-positive RBCs transfusion. This may help to reduce the worry about developing complications when switching Rh/D-negative patients to receive Rh/D-positive red blood cells. The practice will also help in maintaining negative red blood cells unit stock from shortage and save it for needier patients and ultimately improve the blood bank services.

الملخص:

إن نقل المرضى الذين لديهم عامل Rh/D سلبي لتلقي خلايا الدم الحمراء إيجابية Rh/D ضروري في حالات الطوارئ. يتأثر معدل التحصين المضاد لـــ D بعد إعطاء خلايا الدم الحمراء الإيجابية Rh / D للمرضى السلبيين Rh / D حسب العمر والجنس والحالة المناعية وعدد وحدات خلايا الدم الحمراء الإيجابية Rh / D المنقولة والمدة لتكوين الأجسام المضادة. إن التحكم في تأثيرات هذه المتغيرات وفهمها على زيادة فرصة التحصين الخريفي يسمح للأطباء المعالجين بممارسة تبديل مرضى Rh/D السلبيين لتلقي خلايا الدم الحمراء الإيجابية Rh/D بشكل أكثر أمانًا. تم تنفيذ أساليب إحصائية مختلفة للتحليل بأثر رجعي للمراقبة المقطعية على ١٠٦ مريضًا سلبيًا لـــ Rh/D، وكان ٥٠ مريضًا يتمتعون بكفاءة مناعية بينما كان ٥٦ مريضًا يعانون من ضعف المناعة والذين تلقوا نقل خلايا الدم الحمراء الإيجابية لـ Rh/D. تم استخدام الإحصائيات الوصفية لوصف المتغيرات التي تؤثر على معدل التحصين المضاد لـ D. تم استخدام التحليل ثنائي المتغير والجمعيات المحتملة لتقييم الاختلافات بين العوامل التي تؤثر على معدل التمنيع الخريفي بين المرضى. تم استخدام تحليلات Maltivarate لتحديد العوامل التي تؤثر على معدل التمنيع الخريفي. ٦ من أصل ١٠٦ (٥,٦%) من المرضى الذين لديهم عامل Rh/D سلبي تلقوا خلايا دم حمراء إيجابية Rh/D تم تخصيصهم بمستضدات فصائل الدم المختلفة (NSW، Jk^b و4 .(E من ٦ مرضى مصابين بمضادات Rh/D كانوا مرضى ذوي كفاءة مناعية. كان التحصين Alloimmunization أقل في المرضى الذين يعانون من ضعف المناعة فقط ٢ من أصل ٦ مرضى alloimmunzed. هذه الدراسة هي الأولى في المملكة العربية السعودية التي تحدد معدل التمنيع الخريفي لدى المرضى الذين لديهم عامل Rh/D سلبي والذين لديهم حالة مناعية متنوعة، والذين تعرضوا لنقل كرات الدم الحمراء إيجابية Rh/D. قد يساعد هذا في تقليل القلق بشأن حدوث مضاعفات عند تبديل مرضى Rh/D السلبيين لتلقي خلايا الدم الحمراء الإيجابية Rh/D. ستساعد هذه الممارسة أيضًا في الحفاظ على مخزون وحدات خلايا الدم الحمراء السلبية من النقص وحفظها للمرضى المحتاجين وتحسين خدمات بنك الدم في نهاية المطاف.

Introduction:

Transfusions involve transferring suitable donor blood to a recipient. The human blood group system includes ABO, Rh, Kidd, Kell, Duffy, MNS, and Lewis. ABO and Rh are of most clinical importance. Blood transfusions with ABO or Rh/D incompatible blood might cause serious responses. Dr. James Blondell performed the first person-to-person transfusion in 1818 using postpartum hemorrhage patients. Information on blood group systems, components, and storage has grown rapidly since the early 20th century. Transfusion Medicine emerged (Giangrande, 2000). ABO blood groups are the most essential. The short arm of chromosome 9 (9q34.2) contains the ABO Blood Group System gene, which determines whether red cells carry the A, B, or both antigens. Three variations of the gene form four blood groups. Healthy people produce red blood cell antibodies against A or B antigens not expressed on their cells. The majority of natural antibodies, IgM immunoglobulins, assault and kill red blood cells carrying the antigen. Transfusion of ABO-incompatible red cells can cause hemolysis (Johnson, Brown and Slayten, 2018).

After ABO, Rh Rhesus is the second most clinically significant blood group. The Rh blood group has the RH locus on chromosome 1's short arm (p36.13–p34.3). It has RHD and RHCE genes. The RH locus genes stimulate genetic exchange and produce new antigens C, c, E, e, D (Avent and Reid, 2000). Red cells with Rh/D on their surfaces are Rh/D-positive, whereas those without it are Rh/D-negative. Transfusions, pregnancies, and organ transplants expose people to Rh/D antigens, which then develop into IgG immunoglobulins. Haemolytic transfusion responses can result from anti Rh/D antibodies destroying Rh/D-positive red cells (Weinstein, 2016). Rh incompatibly causes Hemolytic Transfusion Reactions (HTR) by extravascular hemolysis of donor RBCs and macrophages in the recipient's spleen and liver. The donor RBCs may be coated with the recipient's antibodies, but these antibodies do not cause intravascular hemolysis. Macrophages destroy them. Fc of this antibody is recognized by macrophage IgG-Fc receptors, aiding donor cell phagocytosis. Rh blood group antibodies cause extravascular hemolysis. On donor RBCs, another antibody may bind C3b without triggering the cascade. Macrophages with C3b receptors phagocytose more. Incorporate ABO, Duffy, and Kidd blood group antibodies (Flegel, 2015).

Rh/D-negative people, especially pregnant women, should not be transfused with Rh/D-positive red cells on the whole. In the absence of Rh/D positive transfusion to Rh/D negative recipients, a haematologist should be consulted and anti-D immunoglobulin administered. World population growth has increased blood transfusion needs. Transfusion medicine allows for many solutions, and allogenic transfusions that switch from recipient blood group to another blood group under certain conditions and strict regulation improve the blood bank's quality and readiness to serve the medical system (AABB, 2019).

Transfusion therapy protocol optimizes blood safety and is crucial to routine red blood cell transfusion management. Rh/D negative patients may need to receive Rh/D positive red blood cells due to a shortage or increased demand in blood banks in large city hospitals and emergency units that treat a large number of patients and perform major operations that require large blood transfusions (Weinstein, 2016).

Aim

Determination of alloimmunization rate with anti-D in Rh/D- negative patients who received Rh/D- positive red blood cells, with regard to patients' immune status, health conditions, age, gender, ABO group and number of RBC units received.

Objectives

1. Determine alloimmunization rate with anti-D after switching Rh/D- negative patients to receive Rh/D-positive red blood cells.
2. To find out the influence of the following variables: gender, age, immune status, number of transfused RBC units and duration of antibody formation on the alloimmunization rate.

Significance of the study:

This study, the first of its kind in Saudi Arabia, will help clinicians manage Rh/D-negative patients who need Rh/D-positive red cells. Retrospective investigations of patients will also inspire future researchers to do prospective studies in this new discipline of transfusion therapy.

Literature Review

Blood Transfusion Compatibility

Blood and plasma from similar ABO and Rh/D groups are recommended for transfusions. If the same blood type is unavailable, the patient may receive a compatible product (AABB, 2019).

Table2.1: ABO& Rh Blood group systems Compatibility.

Blood Type	Compatible RBC Types	Compatible Plasma Types
A	A, O	A, AB
B	B, O	B, AB
O	O	O, A, B, AB
AB	AB, A, B, O	
Rh/D Positive	Rh/D Positive Rh/D Negative	Rh/D Positive Rh/D Negative
Rh/D Negative	Rh/D Negative	Rh/D Positive Rh/D Negative

1) **Transfusion guidelines from the American Association of Blood Banks (AABB):**

Overall, hospitalized patients should use limited RBC transfusion techniques. The intensive care unit may transfuse hemoglobin levels below 7 g/dL. Patients with hemoglobin levels below 8 g/dL or oxygen delivery problems may get transfusions during surgery (AABB, 2019).

In stable inpatient cardiovascular disease patients, transfusions should only be considered at hemoglobin levels of 8 g/dL or less or if oxygen delivery symptoms are present. Symptoms and hemoglobin levels should guide transfusion decisions in hospitalized stable patients (Carson et al., 2012).

To minimize immune-mediated transfusion reactions and alloimmunization, AABB guidelines require the blood bank manager to consult with the physician requesting blood transfusion about the patient's clinical condition, age, gender, and immune status before agreeing to D+ transfusion to D- recipient. The guidelines conclude that more research is needed, particularly clinical trials in cardiovascular disease patients and the elderly and trials testing hemoglobin limits below 7-8 g/dL (AABB, 2019).

2) Transfusion guidelines from the College of American (CAP):

Most recent CAP guidelines focus on surgical, non-surgical, and chronic anemia patients. If hemoglobin is 10 g/dL, these guidelines recommend RBC transfusions. Transfusions for individuals with hemoglobin 6-10 g/dL depend on blood loss and clinical state. Previous immune competent people who lose 30-40% of their blood volume do not need transfusion and should be rehydrated with crystalloids and colloids (College of American Pathologists, 2019).

Based on hemoglobin and underlying conditions, immune compromised patients need RBC transfusion at decreased blood loss volumes. Most patients require RBC transfusion after acute blood volume loss of 40% or higher. According to guidelines, peripheral tissue oxygenation indices should determine transfusion. In ordinary practice, clinical signs and symptoms such heart rate, blood pressure, hemoglobin level, medical history, and bleeding status must be considered. Hypovolemia should be treated first in severe anemic patients, according to CAP guidelines. In major bleeding, it may be best to delay euvolaemia until bleeding is under control (College of American Pathologists, 2019).

Normally, one unit of RBC is replaced by newly generated RBC each week, which can be used to determine RBC replacement needs in patients with different marrow function. Thus, RBC transfusion frequency and quantity should be determined by bone marrow compensation, cardiovascular tolerance of the volume transfused, alloimmunization, scheduling and availability of compatible blood, and blood bank resource conservation (Simon et al., 1998).

3) The Saudi Society of Transfusion Medicine (SSTM):

The Saudi Society for Blood Transfusion's periodic conferences aim to build a national blood system and positively impact donor motivation strategies and planning, standardization of policies and processes, effective use of resources, supplies, modernization and development of blood transfusion services, and exchange of experiences and applied technical methods to improve blood quality.

Rh Blood Group System:

1) **Rh/D Negative Phenotype**

Rd. and Arche proteins have 12 transmembrane domains, intracellular NH2 and COOH termini, and 6 orofacial loops. The D and CE polypeptide and Cc and Eel polymorphism amino acid variant These provide eight frequent Rh System phenotypic combinations (Table. 2.2) (Westhoff, 2007). Rd.-negative people have deleted or deactivated RHD genes (Harmening, D., 2015).

Table 2.2: RH system phenotype, Gene complexes and antigens (Westhoff, 2007).

Phenotype	Genes Present	Antigens Present
R1	RHD Arche	D,C,e
R	RHce	Dce
R^2	RHD RHcE	DcE
Ro	RHD RHce	Dce
r'	Arche	dCe
r"	RHcE	dcE
R^z	RHD RHCE	DCE
r^y	RHCE	Dce

The presence or lack of Rd. protein in the RBC membrane determines Rhesus positivity. An upstream and downstream Rhesus box on two chromosomes recombine to delete the RHD gene (Figure 2.1). Unequal crossover. A D-negative individual is homozygous for the haplotype that lacks the RHD gene (C) at the RH gene location when the two crossing strands separate (from A over the recombination site to B).

2) Prevalence of Rh/D- Negative Phenotype

Europe, Canada, and the US have 15% Caucasians with Rh/D-negative phenotypic, which is rare worldwide. Rh/D-negative phenotypes are most common among Basques and Pyrenees (25–35%) and Imazighen (18–30%) (Flegel, 2007). Only a few research has examined the prevalence and distribution of ABO and Rh blood group phenotypes in Saudi Arabia. Owaidahet al. (2020) examined the major blood group antigen frequencies in Eastern KSA. The research of 100 Saudi blood donors revealed that 80% were Rh\D-positive and 20% were Rh\D-negative. Elsayidet al. (2017) reported 13.7% Rh/D-negative phenotypes among 600 Saudi donors in Riyadh. Alabdulmonemet al. found ABO and Rh seroprevalence in Buraidah, Qassim blood donors. This study found 89.8% Rh-positive donors and 10.1% Rh/D-negative donors among 4590 blood donors (781 females and 3809 men) (Alabdulmonemet al., 2020).

Alloimmunization:

Alloimmunization is the generation of immunity to foreign antigens by exposure to genetically distinct cells or tissue from the same species. This is especially important for chronic blood transfusions. Blood transfusions introduce foreign antigen cells that last various amounts of time. 2002 (Daniels et al.).

In the latest ISBT updates on August 6, 2019, 38 blood group systems, 45 genes, and 346 antigens were approved. After blood transfusion, recipient blood groups trigger the immune system to respond to donor antigens. In line, this will have different clinical repercussions based on the blood cells and specific antigens, such as human leukocyte, granulocyte, platelet, and RBC antigens (Douglas Blackall, 2017).

Transfusion responses against RBC antigens are the most common. RBCs have clinically important antigens D, E, e, Kell (K), Duffy (Fy), and Kidd (JK). Surface protein antigens produce warm-reacting IgG alloantibodies, which interact with RBCs to cause hemolysis at 37°C in transfused patients (Mitra, Mishra, and Rath, 2014). Some transfusion responses, such fever non-hemolytic transfusion reaction (FNHTR), are minor and resolve on their own, but others, like acute hemolytic transfusion reaction, caused severe morbidity and mortality (Strobel, 2008).

Well-known is that anti-D antibodies can cause Hemolytic Transfusion Reactions (HTR) and severe fetal and newborn hemolytic disorders (HDN) (Johnson, Brown and Slayten, 2018). Sharing ABO and Rh types is the golden rule of blood transfusion. In some situations, Rh/D-negative individuals may need D-positive red blood

cell transfusions due to a shortage of RBC units. Especially when blood banks are short or in high demand for Rh/D-negative RBCs. City hospitals and emergency units that treat many patients or do significant surgeries that require many blood units typically experience this (AABB, 2019).

1) Recipients Immune System Conditions:

Every transfusion exposes recipients to many foreign non-selfblood group antigens of the donor. There is number of factors affecting recipient immune system and enhance alloimmunization. (Figure 2.2) Shows some of recipients conditions that may influence the chance of alloimmunization between alloimmunized and non-alloimmunized patients (Hendrickson, 2018).

Figure 2.2- Alloimmunization in Alloimmunized and Non-Alloimmunized Patients

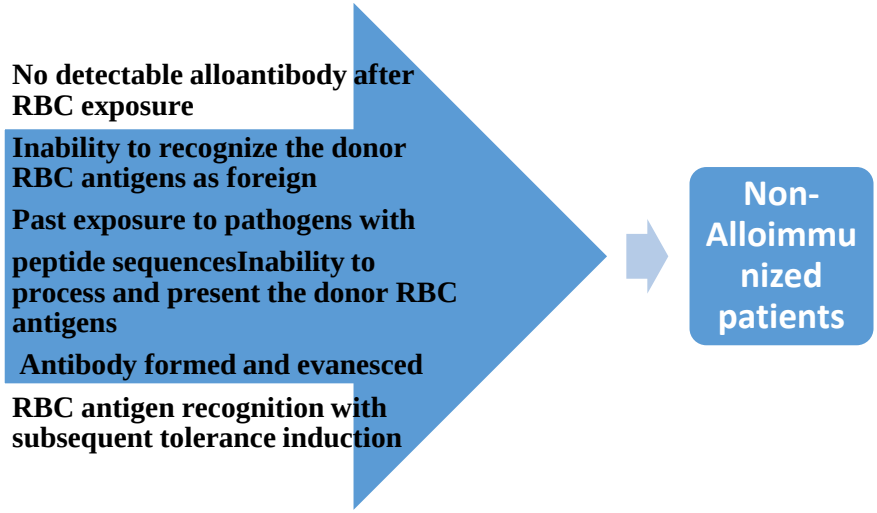
Hemogloinpathies thalassemia and sickle cell disease patients produce alloantibodies due to prolonged transfusion, hence most research of alloimmunization rate have focused on them. However, blood group antigen dominant peptides are crucial to understanding transfusion reactions and alloimmunization. Alloimmunization rate investigations in sickle cell disease patients identified large quantitative variations in B-cells and T-cells, some memory T-cell (CD4+) populations, and modest toll-like receptor expression changes. (2015) Vergertet al. Due to monocyte hemeoxygenase changes, T-cell polarization differs (Zhong et al., 2014).

2) Factors Influencing Alloimmunization Rate:

Alloimmunization from blood transfusion depends on more than antigen immunogenicity. A number of studies examined the factors that affect RBC antigen alloimmunization frequency.

Age, gender, ethnicity, recipient immunological state, clinical conditions, and blood transfusion frequency were examined in such investigations. Patients with antibodies are more likely to develop antibodies. Alloimmunization risk increases with additional transfusions. Thalassaemia and sickle cell anemia patients, who need RBC transfusions frequently, are badly affected by this. These individuals had a 25% greater alloimmunization rate than non-transfusiondependant patients (Zimring et al., 2011). Studies showed this. Lal et al. (2018) surveyed 717 US individuals with thalassaemia major who received chronic and intermittent transfusions. From 53 of 314 (16.9%), 85 antibodies against RBC antigens were identified. It had 71 alloantibodies and 14 autoantibodies. Out of 314 patients, 39 (12.4%) had 60 alloantibodies. Around 65% of significant alloantibodies were against Rh (21/60) and Kell (19/60) blood groups. Vichinskyet al. examined transfusion-related problems in a US cohort of 407 thalassaemiamajor patients. Here, 19% (68/365) of patients acquired alloantibodies. Chronically transfused patients had 23% alloimmunization compared to 13% for intermittents. Anti-E, anti-c, anti-Jk, anti-Jk, anti-Kell, anti-S, anti-f caused most hemolytic transfusion responses. In addition, age, race, and splenectomy status impacted alloimmunization rate (Vichinskyet al., 2014).

There were 184 various surgeries, 95 hamate-oncology cases, 19 cardiology cases, 13 nephrology cases, 13 gastroenterology cases, and 19 other disorders in the control group. Two-thirds of red cell transfusion patients



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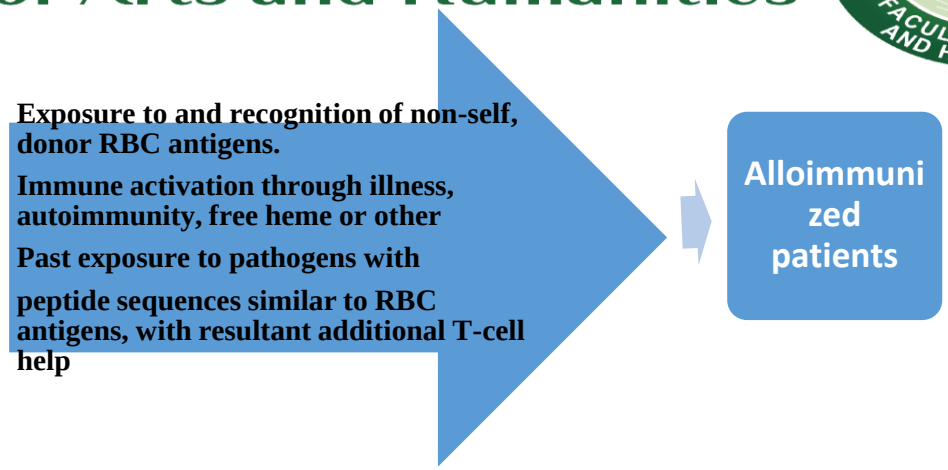


immunomodulatory medication, and 19% needed mixed immunomodulation. 10 of 119 patients had further red cell alloimmunization, compared to 12 of 357 in the transfused sex-matched control group with non-inflammatory diseases. The control group had more transfusions than IBD patients. In addition, only 1.4% of pregnant IBD patients had antibodies. As most vaccinated IBD patients were young, alloantibody-related hemolytic sequelae had high lifetime risks. Finally, IBD patients had a higher risk of transfusion-induced alloimmunization, possibly due to inflammation. They received less RBC units than the control group, yet this happened (Papay, P. et al., 2012).

3) Historical Studies Dealt with Immunizing Healthy Volunteer with D Antigen

We know that Rh/D-negative moms must use anti-D serum during birth to avoid D antigen vaccination. Weakly responding anti-D women can receive Rh/D-positive RBCs. Produce high-titre anti-D serum. Gunsonet al. established an immunizing program to detect the best way to produce high-titre anti-D serum by immunizing Rh/D-negative male volunteers with washed O R2R2 cells from Rh/D-positive donors. Since Group O R2R2 red cells had highly reactive D-antigen relative to other Rh genotypes, an immunizing donor with this group was chosen (Gunsonet al., 1970).

Weakly responding Rh-antibodies were investigated using antiglobulin stacking. Separations using ultracentrifugation and chromatography were used to determine antibody types. Every group had one person who did not respond to sensitization. As for the remainder, antibodies appeared in the blood 37 to 130 days later. Along with Anti-D, two volunteers had anti-E. Both 2 and 7 have anti-E after 44 days of anti-D. 14 days following its emergence, anti-D was strongest. Other than volunteer 10, all others acquired 7SyG and 9SyM immunoglobulin. Out of 11 participants, nine responded to immunization, and both groups (dosage of 5 ml and 0.5 ml) had anti-D in their blood after 14 days of sensitization. Thus, 0.5 ml sensitizes the individual to anti-D development, but not everyone. Detection of Anti-D in the blood took 37 to 130 days after inoculation, proving that antibodies are formed via secondary reactions. Due to variables like extended survival of Rh/D-positive RBCs in Rh/D-negative individuals and slow phagocytosis of foreign cells, antibodies are formed later (Gunsonet al., 1970).

4) Studies Dealt with Frequency of Alloimmunization in Immune Competent Status

Radkay, Triulzi, and Yazer examined hemolysis, an early sign of alloimmunization after uncross matched red blood cell transfusion. Unexpected clinically significant antibodies cause hemolysis after uncross matched red blood cell transfusion. The blood bank can provide cross matched red blood cells within 60 minutes after receiving the patient's blood sample. This little delay in RBCs can be life-threatening in simple cases. Uncross matched RBCs can be given immediately for acute recovery while the blood bank does antibody screening. They examined transfusion service database records of uncross matched red blood cell recipients over 9 months to assess hemolysis risk. Each recipient's basic statistics and number of uncross matched RBCs were gathered. To determine if hemolysis occurred after uncross matched RBC unites transfusion, history and laboratory hematological and biochemical data were examined for cases with clinically significant antibodies or those who developed them on the day of transfusion.

Frohn et al. examined the likelihood of anti-D development in 78 Rh/D-negative (ccddee) patients who had Rh\D-positive RBCs tranfusion in Germany. Patients had abdominal surgery (42), cardiac surgery (33%), trauma (14%), miscellaneous (6%), and disseminated intravascular coagulation (5%). Hematological patients and women under

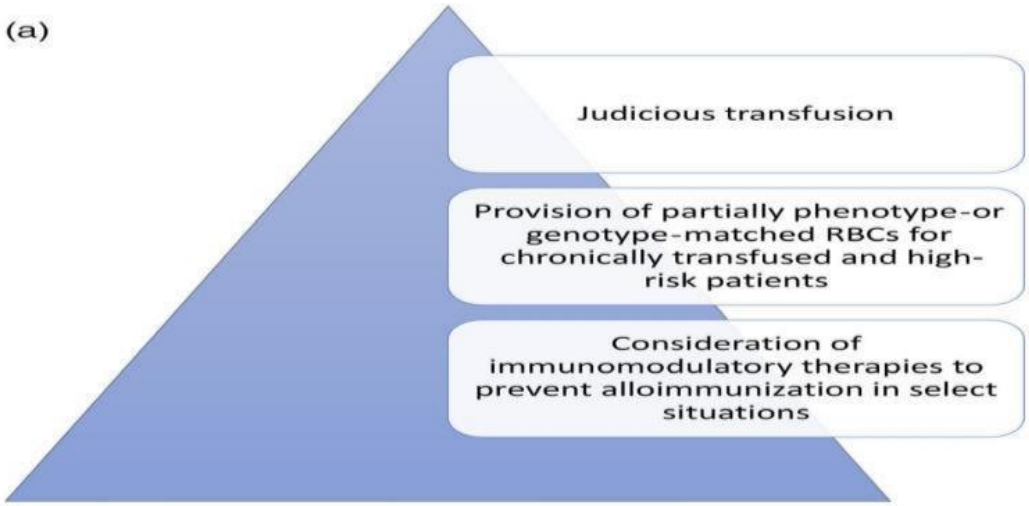
40 were not commuted and received no RhIG. Antibody-screening tests were performed between 14 days and 1 year before conversion. The doctors were asked to look for hemolysis.

According to Frohn et al., the frequency of anti D after switching Rh/D-negative patients who received Rh/D-positive RBCs is much lower than the figure quoted in current textbooks, which was based on the initial study on Rh-D-negative healthy volunteers who were exposed to Rh-D-positive RBCs. However, Frohn et al. supported these findings with other study settings and historical volunteer investigations. The test detection methods were more sensitive than before. DAT was always conducted to detect cell-bound antibody before serum appearance. In earlier investigations, Rh/D-positive RBC units were repeatedly donated to improve immunization and generate the most anti-D for medical use, but in the present study, patients got only one blood transfusion. His patients' health status may also explain why they never received immunosuppressive drugs, but rather stress-induced immune suppression. The antigenic stimulus in the patients lasted for weeks, but the immune system recovered and should have normalized. The risk of generating anti-D after D-mismatched RBC transfusion is much below the predicted range, according to Frohn et al. (2003).

Yazer and Triulzi examined anti-D detection in Rh/D-negative receivers transferred with Rh/D-positive red blood cells. Transfusing Rh/D-positive blood into oncology patients has been shown to reduce alloimmunization. Alloimmunization rates of non-oncology Rh/D negative recipients transfused with Rh/D positive RBCs were examined. By studying Rh/D-negative receivers who were not alloimmunized to Rh/D antigen and had a follow-up antibody screening 10 days following their first Rh/D-positive RBC transfusions. Data was acquired from transfusion service records on recipients' age, sex, number of Rh/D positive RBC transfusions, immunological status, serologic investigations, and hospital ward. The emergency department, operating room, intensive care unit, or medicine ward received 82% of Rh/D-positive units in the sample. Hematology-oncology wards received no RBC units. In 98 Rh/D-negative immune competent receivers who received Rh/D-positive transfusion, 24 generated 44 new alloantibodies: 22% anti-D, 11% anti-E, 5 anti-C, 2 anti-K, and 1 each of anti-Kpa, anti-Jk, anti-Bg, and anti-Fyb. In immunological competent patients, 22% were alloimmunized with anti-D (Yazer and Triulzi, 2007). We will discuss the alloimmunization rate of Rh/D negative oncology patients transfused with Rh/D positive RBCs in a special section of the literature review, taking into account the different diagnoses and alloimmunization rates.

5) Prevention of Alloimmunization Complications:

Judectively transfusing improves transfusion techniques and reduces alloimmunization problems. Prudent transfusion involves following guidelines, indications, and switched Rh/D transfusion components. Reducing alloimmunization is the best strategy to avoid issues. (2.3 a). 2020 (Hendrickson).



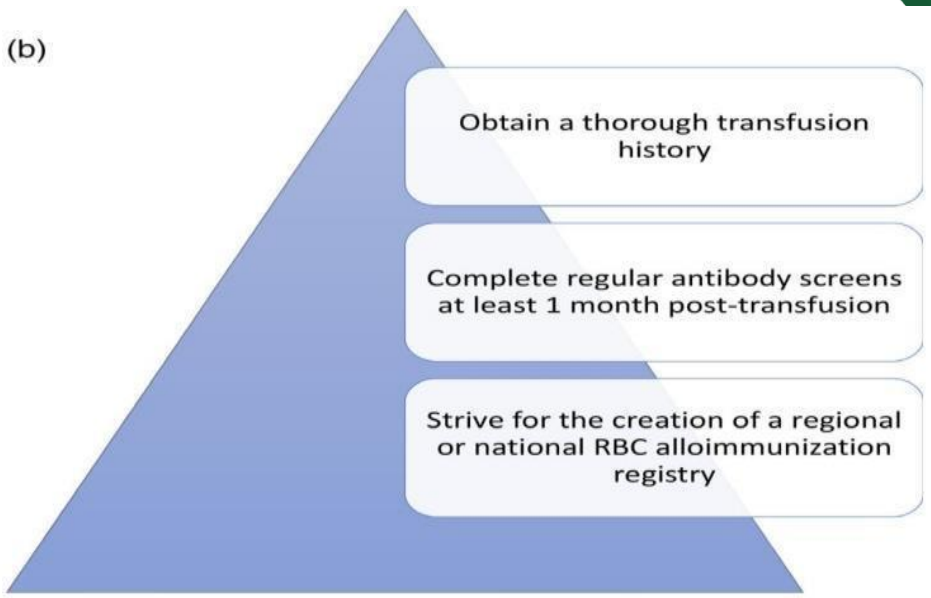


Figure2.3: (a) Strategies to Decrease Alloimmunization (b)Recognizing of Pre-Existing Alloantibodies (Hendrickson, 2020)

Wider patient blood management options include recognizing preexisting alloantibodies for transfusions. Figure 2.3b Partial phenotypic and genotypic matching can reduce alloimmunization rates and transfusion rates without affecting patient treatment or results (Compernolleet al., 2018). All these research indicated distinct differences in alloimmunization rates among patient groups and how to reduce clinical hazards. Studies are needed to determine if some patients need a less restrictive D mismatching transfusion protocol.

Materials and Methods:

1) Patients and Study Setting:

In Saudi Arabia, the 1,600-bed King Faisal Specialist Hospital and Research Centre (KFSH & RC) in Riyadh and Jeddah conducted the study at its Blood Bank (Hematology Department). Inpatients and outpatients get primary care at this hospital and research institution, which participates in many international and national clinical trials. The hospital specializes in organ transplantation, oncology, cardiology, neurology, and genetic problems, making it one of the best in Saudi Arabia. Outpatient visits average 10,000 per year, and 28 local hospitals cooperate with the facility. Data will be kept confidential after this thesis was approved by the Department of Clinical Laboratory Science Council and the research ethics committee of King Faisal Specialist Hospital and Research Centre (KFSH&RC). Scientific research committee IRB RP20-6 approved this thesis (Appendix 1).

2) Study Design

This observational cross-sectional investigation tested anti-D alloimmunization prevalence. Understanding risk factors and variables that boost alloimmunization after receiving Rh/D-positive red blood cells (RBCs) from Rh/D-negative individuals and how this probability relates to clinical condition and immune status.

3) Sample Size and Data Collection Method:

At King Faisal Specialist Hospital and Research Centre, patient data is obtained via blood bank records in the EHR and consultation reports. (Appendix2). Data was collected from 2013 to 2021. 65 Riyadh and 41 Jeddah patients were recorded.

Inclusion and Exclusion Criteria:

1. D- negative patients who were not alloimmunized to the D antigen and who have post-transfusion antibody screening results after receiving D- positive RBC transfusion in the time interval of 14 to 60 days.
2. No RhIG was given in any case.
3. Availability of all information of patients' variables that affect alloimmunization.

4) Equipment and Material

As shown in (Figure 3.1), the following Quality Assurance protocol of pre-transfusion testing in the blood bank department of King Faisal Specialist Hospital and Research Centre (KFSH&RC) was applied to each sample, and

positive and negative controls were included with every test. At least once a day, automated system controls and timings were set. Controls were done at reagent lot number change and establishment. Control samples were loaded similarly to test samples. When controls don't react as predicted, investigations were done to find the cause and confirm all tests after the most recent legitimate control findings. Using the following materials and equipment for pre transfusion compatibility testing:

1. Incubation
2. Centrifugation
3. Normal Ionic Strength Saline (NISS) to prepare Red Cell Suspensions
4. Microcolumn Gel cards ABO Grouping, Rh Typing & Antiglobulin Tests
5. Red Cell Antibody Screening & Identification

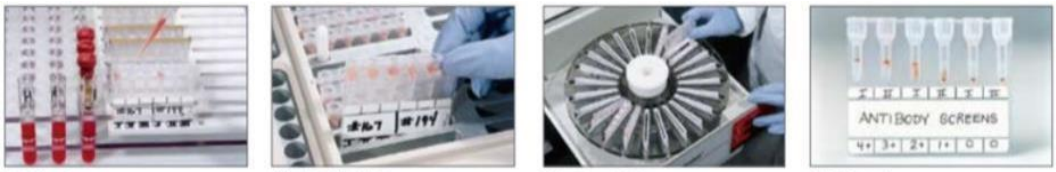


Figure 3.1: Gel Column Agglutination Processing

Blood sample:

3 ml of EDTA-Ethylene diamine tetraacetic acid samples not hemolyzed, lipemic, or icteric samples, stored at 2 to 8 °C for maximum for 7 days. Donor blood in SAGAM saline-adenine-glucose-mannitol tested during the expiration date of the unit when stored at 1 to 8°C. Separation of RBCS and plasma by centrifugation at 10 minutes at 2000 rpm. Donor segments do not require centrifugation. Using Column agglutination techniques with several diverse commercial cards/cassettes and cell panels (Ortho clinical diagnostics). Cards consist of monoclonal antibodies for direct ABO testing and Rh phenotyping. Cell panels include blood group antigens for antibodies screening and identification. The reactions were read using Gel Column Agglutination.

Laboratory Methods:

1. Gel Column Agglutination ABO direct/reverse grouping and D grouping:

Using blood grouping card DG Gel ABO/Rh™ REF 210384 (Appendix 2) ABO direct/ reverse grouping and D grouping card consists of six microtubes containing monoclonal anti-A and anti-B reagents in direct ABO grouping. A 1 and B cells are used for reverse grouping; group. Monoclonal anti-D reagents will allow detection of most D variant expressions with this reagent although eaction strengths may vary.

2. Gel Column Agglutination Complete Rh phenotyping:

Using IH-Card Rh-Phenotype™ REF 813202100 (Appendix 3) Rh-Phenotype card consists of six microtubes containing monoclonal IgM Anti-C, Anti-E, Anti-¢, Anti-e, Anti-K and Control.

3. Pre-Transfusion Antibodies Screening:

Using the ID-Micro Typing system from Ortho Clinical Diagnostics™. RBCS Reagent derived from three or two single group O blood donors, correspondingly, in separate vials for the detection of red blood cell antibodies. Including the following antigens: D, C, E, c, e, K, k, Fya ,Fyb , Jk , Jk , M, N, S, s, Lea , Leb , P1, Xga . All vials contain red blood cells which are negative for the following low-incidence blood antigens: Jsa ,Kpa , Wra , Dia , Vw, V, Lua and Cw. The specifications for the vials as following vial1 R1R1 or R1 w R1, vial 2 R2R2 and vial 3 rr. Double dose expression of the following antigens: M, N, S, s, Fya, Fyb, Lea, Leb, Jk and Jk and expression of Lub, Kpb, Xga and Coa.

4. Gel Column Agglutination Direct Anti-Human Globulin:

Using DG gel anti-Ig-G REF Card™ (Appendix 4) Anti-IgG consists of six microtubes containing a gel impregnated with rabbit polyclonal Anti-Human Globulin Anti-IgG that does not contain antibodies to complement components. The Anti-IgG is light chain specific and thus may also agglutinate IgA or IgM coated RBCs. The Anti-IgG is diluted

in a phosphate buffered saline solution containing bovine albumin, absorbed to remove heterospecific antibodies and contains a mixture of colorants Patent Blue and Tartrazin.

3. Antibodies Identification:

Using ID-Micro Typing System™ Anti-IgG, -C3d Card. RBCs Reagent with polyvalent antigens of eleven single blood donors in separate vials for the identification of RBCs antibodies. Panel of papain treated 11 RBCs Reagent with polyvalent antigens of eleven single blood donors, in separate vials. Panel 11 contain the following antigens: D, C, E, c, e, K, k, Fya, Fyb, Lua, Lub, Jk, Jk, Jsb, M, N, S, s, Lea, Leb, P1, Xga, Coa and if available: Jsa, Dia, Cw and Kpa. Panel 11 Papain contains the antigens: D, C, E, c, e, K, k, Lua, Lub, Jk, Jk, Lea, Leb, P1, Coa, and if available: Jsa, Di a, Cw and Kpa

Statistical Analysis

Researcher Hypothesis H1 supposes that there is a relationship between immune status, gender, age, ABO blood type, frequency of transfusion, duration of antibody formation, and alloimunzation with Rh/D antigen. Null hypothesis H0, supposes that there no relationship or association between variables. To examine the Researcher hypothesis, we run hypothesis tests (Chi-squire in case of categorical variables, and Independent T test for case numerical test). If the Probability calculated P value is less than 0.05, then we consider H1 is correct and H0 will be rejected and vice versa.

Data cleaning:

Reports will be collected then coded and revised, and data will be introduced on statistical software IBM SPSS version 26. All statistical analysis will be done using two-tailed tests and an alpha error of 0.05. A P-value less than 0.05 will be considered statistically significant.

Sequence of Planned Analyses:

1. The data were analyzed using SPSS v.26, then described according to their values proportion in (Age, Gender, Blood Group and Immune Status) within variables (Gender*Age Group), (Gender* Alloimunzed Patients), and (Blood Group* Alloimunzed Patients).
2. The age was grouped into 5 intervals with width equal 20. The statistical of the Number of Transfused Blood Units and Duration of Antibody formation were determined. The rate of allo-immunization was determined.
3. The association between nominal variables (Gender, Age Group, Immune Status) and antibody formation was tested using chi-square at level of confidence 95% (P.value 0.05).
4. The differences in the statistic mean of transfused blood units between group 1(Alloimunzed Patients) and group 2(Non Alloimunzed Patients), were tested using independent T test at level of confidence 95% (P.value 0.05).
5. The differences between duration of antibodies formation statistic mean between blood groups were tested using one-way ANOVA test, at level of confidence 95% (P.value 0.05).
6. The relation between (Gender*Immune Status*Blood Group) and (Number of Transfused Blood Units*Duration of Antibody Formation) were tested using multivariate test, at level of confidence 95% (P.value 0.05) (Warner, 2007).

Results

These results obtained using study tools according to the study hypotheses and analysis of different variables to achieve the objectives of the study. First, descriptive analysis of the variables will be presented then analysis of variables affect alloimmunization rate will be shown. The present cross-sectional study was conducted in order to examine alloimmunization rate in Rh/D-negative patients. Number of 286 Rh/D- positive blood units were transfused to 106 Rh/D- negative patients during period between 2013 to 2021 in the blood bank of KFSH&RC in Riyadh and Jeddah region. 6 out of 106 (5.6%) Rh/D-negative patients received Rh/D-positive red blood cells were alloimmunized with different blood group Antigens (NSW, Jk^b and E). 4 of 6 alloimmunized patients with Anti Rh/D were immune competent patients. Alloimmunization was lower in immune compromised patients only 2 out of 6 alloimmunized patients.

Summary of Total Patients' Descriptive Data:

Patients have mean age of 51± 22 years. 55.7% of patients were male, while female comprised 44.3% of the study populations. 47.2% where immune competent while 52.8% were immune compromised. The patients were admitted to the hospitals where the study conducted, for different pathological conditions including: Cardiac surgery (35.3%),

oncology (23.2%), post-transplant (14.1%), liver disorders (9.8%), general surgeries (8.7%), cardiac diseases (6.6%), renal diseases (5.5%), Rheumatic diseases (3.3%), infections (2.2%), and accidents (1.1%). as shown in (Figure 4.1). According to exposure to switched transfusion, O blood group was the most exposed to transfusion, with a percentage of 62.3%, followed by B blood group 18.9% then A blood group 17.9% while the least exposed to transfusion was AB with a percentage of 0.9% according to the prevalence rate of each group. The main number of transfused units was 8 ± 13. The main frequency of transfusion 2.7± 3. The main Duration of antibody formation 56± 101 days. Characteristics of the studied population are summarized in (Table 4.1).

Conclusion:

Alloimmunization is a common transfusion associated complication. However, it is not the normal outcome of a transfusion as most recipients never become alloimmunized. Currently, it remains uncertain whether non alloimmunized patient are tolerated to all or some of antigens that they have previously been exposed to, or whether their immune systems are simply unaware of the antigenic exposures. In this regard, understanding non alloimmunized patient may be equally as useful as understanding alloimmunized patient. However, alloimmunization after switched transfusion will remain a clinical problem in the years to come, because of widely variation phenotypic or genotypic ally matched RBCs between patients and recipient is simply not practicable. Alloimmunization is affected by different variables like immune status, gender, age, ABO blood type, frequency of transfusion, duration of antibody formation. Understanding the impact of the variables that affect alloimmunization will make the switched transfusion process safer for the patients and will give the blood bank the confidence and courage to take decision that benefit both the patients and the blood bank inventory. To reduce alloimmunization include judicious transfusion that consider all variables that affect alloimmunization as well as the selection of partially antigen matched RBCs for patient populations at highest risk for alloimmunization. Pre-transfusion antibodies identification test to detect the dangers of existing alloantibodies include regular planned post-transfusion antibody screens, obtaining a thorough history including past transfusion and recent transfusions.

Recommendations:

1. Raising awareness of Saudi communities on the necessity of blood donation, its importance and benefit through various media.
2. Training and continuing education for specialists working in the field of blood transfusion and blood banks on the optimal uses of blood products to ensure that units are delivered to the best beneficiaries.
3. Digital transformation of all patient data to electronic files of the digital system for health institutions and working to link digital systems of all health facilities on one platform which facilitates data collection to conduct cohort studies.
4. Continued studies of immune responses to antigens on transfused RBCs, both at the bedside and at the bench, are essential to improving transfusion safety and advancing scientific knowledge.
5. Further studies examining larger sample size in a prospective design will clarify whether a switched transfusion is justified. especially for male patients and women of non-childbearing age for whom the possibility of antibody formation would not have consequences.
6. Research sponsors directorate in King Saud University research centers has to encourage the researchers to study alloimmunization on large scale in a well-designed study including patients' different clinical cases, and to include factors known in literature to influencing alloimmunization rate

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