

## Prevalence of red cell alloantibodies among multi transfused dependent thalassemia patient in the Malaysian state of Penang

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**Abstract:** Introduction: Thalassaemia is a common haemoglobin disorder in Malaysia. Regular blood transfusion is life saver for thalassaemia patients and is the main treatment for severely affected patients. These transfusions may be associated with complications such as production of red cell alloantibodies and autoantibodies. The purpose of this study was to determine the prevalence of red blood cell antibodies in our institutions, it's the types and the factors that contribute to it are the development. Methods: A total of 173 thalassaemia patients in Penang who received regular blood transfusion were included in this study. Clinical data was collect and analysed retrospectively. Then the antibodies were identified in patients with positive antibodies screening results by using identification panel of red cell. Result: In 173 patients with thalassaemia, 18 (10.59%) had developed alloantibodies. Seventeen (94.4%) of these patients developed anti-E, while 4 patients had developed anti-c (22.2% ), Two had anti-e and anti-Jk<sup>b</sup> (11.1%), and one patient developed anti-Fy<sup>b</sup> (5.56%). Conclusion: This study showed that the specificity of alloantibody detected around the world was uniform with the most common encountered antibodies being directed against Rh blood group system. Based on finding supported in previous studies, it is suggested that extended matching of packed cell for minor antigen especially Rh-related antigen should be considered by blood services.

**Key words:** Thalassaemia; Alloantibody; Transfusion

### 1. Introduction

The thalassaemia syndrome is a diverse group of genetic blood diseases. Thalassaemia is the most common inherited single gene disorder in the world. The thalassaemias are a heterogeneous group of genetic disorders resulting in the reduced rate of production of one or more of the globin chains of haemoglobin (Haslina et al., 2007; Sadeghian et al., 2009). They are widely distributed worldwide, and more prevalent in certain areas especially among Asians as well as in the middle Mediterranean, Middle Eastern and Far Eastern populations. Due to large population movements, people of Asian descent constitute the majority of patients in many thalassaemia centers in Western countries (Singer et al., 2000). A reduction in the synthesis of the  $\alpha$  globin leads to  $\alpha$  thalassaemia and a reduction in the  $\beta$  globin synthesis results in  $\beta$  thalassaemia as opposed to the approximately equal  $\alpha/\beta$  globin chain synthesis in a non-thalassaemic individual. In Malaysia, HbE and  $\beta$  thalassaemia are the most common inherited haematologic disorders affecting beta-globin (Hassan et al., 2004). Severe thalassaemia patients depend on regular monthly blood transfusion. Blood transfusion is the life saver of severe thalassaemia patients however; this is

associated with complications of blood transfusion caused by the formation of alloantibodies and autoantibodies against red blood cell antigens by the patients. This type of sensitization results in difficulty in obtaining compatible blood, reactions to transfusions, haemolysis and occasionally life threatening adverse events (Singer et al., 2000; Ameen et al., 2003). Haemoglobin is the part of red blood cells that transport oxygen from the lungs to other parts of the body. Due to a lack of normal red blood cells, patients look pale because of a low haemoglobin level that causes anaemia and suffer symptoms such as fatigue and shortness of breath. The red blood cells of thalassaemia patients are unable to provide enough oxygen to the body cells and consequently cells that do not have enough oxygen supply will fail to function effectively thus weakening their bodies and results in proneness to other of diseases. Thalassaemia patients usually have very low levels of haemoglobin of 4-5g/dl compared to the normal level. The normal value for adult male is between 13.8 and 17.2 g/dL and normal level for a non-pregnant adult woman is between 12.1 and 15.1 g/dL.

Lastly the normal level for a pregnant woman is at or above 11.0 g/dL (World Health Organization, 2011). In the absence of stem cell transplantation, thalassaemia is managed by lifelong red cell transfusion. The transfusions are done every 3 to 4

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weeks, with a goal to correct the anaemia, to significantly suppress the hyperactive erythropoiesis, and to inhibit gastro-intestinal iron absorption (Hassan et al., 2004). This regular monthly blood transfusion is performed in order to maintain the mean haemoglobin level of 10-11 g/dL (Noor Haslina et al., 2007).

Regular blood transfusions are crucial to maintain growth and development during childhood and also to sustain good quality of life during adulthood. Although blood transfusion is a lifesaver for thalassaemia patients, it is also associated with some complications such as iron overload, fractures, platelet and red blood cells immunization (Sadeghian et al., 2009; Bashawari et al., 2005). The development of anti-red blood cell antibodies; alloantibodies or autoantibodies unfortunately can significantly complicate transfusion therapy (Singer et al., 2000). The frequency of alloimmunization among thalassaemics ranges from 3.7% to 30% worldwide (Poole, 2004) and the rate of alloimmunization to minor blood group antigens occurs in about 20% to 30% of patient while other research reported the frequency has varied from 5-10% to 19-25% and 30%. The different reports associated the frequencies of alloimmunization to age, number of transfusions and the age when transfusion started. It was shown that the frequency of alloimmunization was significantly lower in patients who were less than 5 years of age when blood transfusion started (Bashawari et al., 2005)

In addition, the factors for alloimmunization are complex and involve at least 3 main contributing elements: the RBC antigenic difference between the donor and the recipient, the recipient's immune status and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system (Noor Haslina et al., 2007). As blood is routinely matched with respect to major blood group antigens; ABO and Rh D antigen, there is a high probability that the donor will have minor blood group antigens not present in the recipients which will result in alloimmunization. Alloimmunization significantly concerns the Rhesus, Kell, Duffy and Kidd system which are clinically significant. They may cause haemolytic transfusion reactions while others are clinically insignificant (Bilwani et al., 2005).

## 2. Materials and method

This cross sectional and prospective study was done with the aim to look into the prevalence of alloimmunization in multi-transfused thalassaemia patient. Blood samples were taken from 173 thalassaemia patients at Seberang Jaya Hospital after ethical approval by Ethical Committee and informed consent obtained from patients. Laboratory work will be done at University Technology Mara (UiTM) laboratory works is from March 2012 until May 2012. A total of 173 thalassaemia patients who receive regular blood transfusions were included in this study. Clinical and serological data were collected and

analyzed. The patient's demographic data were recorded, time interval from the start of transfusion, ABO and Rh blood group, history of splenectomy and the units of blood received were recorded. The samples of blood will be allowed to clot; serum was separated and stored in labeled test tubes at -70°C until the tests are performed batches. After the centrifugation serum was separated and screen by standard blood bank procedure for detection antibodies. An antigen panel is used for antibody screening procedure to detect the unexpected antibodies in the patient's serum or plasma. There are capable detecting clinically significant red cell antibodies by haemolysis or haemagglutination at room temperature and 37° for 15 minutes. Antibody was detected by performing the Low Ionic Strength Solution Indirect Anti-Globulin Test (LISS-IAT) method. Indirect Anti-Globulin Test detects antibodies against red blood cells (RBCs) that are present in the patient's serum.

Finally the alloantibodies were identified in sera of patients with positive antibody screening test using a panel recognize blood group antigens. Samples with positive antibody screening test or have incompatibility of crossmatch, the antibody identification will be performed by using reagent red cell panel that will cover all the significant antibodies. Usually this panel that are form with 11 of panel for identify antibody the antibody that is present. Alloantibody was detected by performing the Low-Ionic Strength Solution-Indirect Anti-Globulin Test (LISS-IAT) method. Indirect anti globulin test detects antibodies against red blood cells that are present unbound in the patient's serum. Results from patients were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0. Chi-Squares and Mann-Whitney U was used for data analysis. A p value of less than 0.05 was considered to be statistically significant.

## 3. Result

The studies are evaluated from 173 thalassaemia male and female patients. They were patients from Hospital Seberang Jaya, Malaysia who received blood transfusions. From the total of 173 thalassaemia, 94 (55.29%) were males while 79 (46.47%) were females. In order to see whether multiple transfusions of thalassaemia patients will contributes to alloimmunization. The range of packed cell transfused to patients in this study is between 10 to 120 units. The majority of the patients (55) (32.35%) had received 10-20 units packed cells.

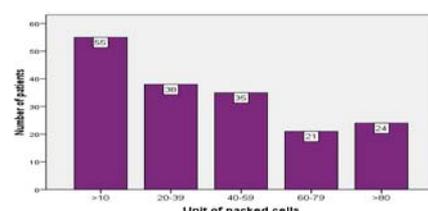
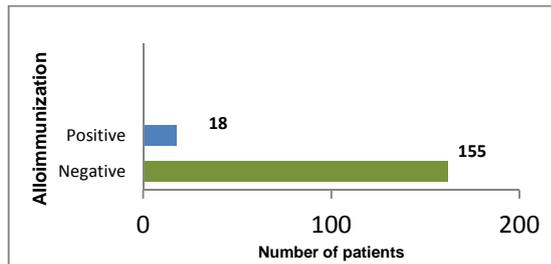


Fig. 1: Unit of packed red cell transfused to patients

The Fig. 2 shows the formation red cell alloimmunization in multi-transfused thalassaemia patients. 18 out of 173 multi transfused thalassaemia patient was positive alloimmunization.



**Fig. 2:** Frequency of red cell alloimmunization in thalassaemia patients

The Table 1 shows the antibodies detected such as anti-Jk<sup>b</sup>, anti-e, anti-E, anti-c and anti-Fy<sup>b</sup> in 18 multi-transfused patients. Anti-E with 94.44% was most detected in thalassaemia patients. Furthermore table 4.3 shows patient number 65 with multiple alloantibodies such anti-E, anti-c, anti-Fy<sup>b</sup> and anti-e and patients with number 162, and 163 with 3 alloantibodies such anti-E, anti-c and anti-Jk<sup>b</sup>.

**Table 1:** Distribution of Various Anti-Red Cell Alloantibodies in 18 Multi-Transfused Thalassaemia Patients

Antibodies Detected	No of Patients	Percentage
Anti Jkb	2	11.1%
Anti-e	2	11.1%
Anti-E	17	94.44%
Anti-c	4	22.22%
Anti-Fyb	1	5.56%

The chi-square test showed significant different association with compare between gender (male and female) and group (group 1= without alloantibody, group 2= with alloantibody) (p<0.05) and Mann-Whitney U test showed no significant differences (P= 0.142) observed between number of packed cells transfused and group of patients (Group 1= without alloantibody, Group 2: with alloantibody).

#### 4. Discussion

In this study, blood sample from 173 multi transfused thalassaemia patients were obtained from Hospital Seberang Jaya, Pulau Pinang. The entire sample had previously been tested for ABO and Rhesus type. Patient demographic data and record of blood transfusion were also obtained from the records of the Hospital Seberang Jaya, Pulau Pinang. Majority of the patients participate in this study were Malay, followed by Chinese, Indian and other ethnic group. This result may reflect the distribution of the races in the country. In Malaysia especially, and in Peninsular Malaysia specifically, the population of Malay in this area is higher when compare to other races such as Chinese and Indian.

Antibody screening test were carried out on the patient serum sample. Three panel screening cell were used for screening. Any blood sample tested positive by screening were then tested for the specificity of alloantibody using an 11 cell panel. Antibody detection and identification are fundamental to the practice of immunohematology. Antibody identification can be a guide to the clinical significance of the antibody and provides beneficial information that aids in the selection of suitable blood for transfusion, but in some circumstances it can be a difficult and time consuming process that causes a delay in a patient care. It has been practiced worldwide that blood banks only provide ABO and Rh (D) against matched blood. For patients who need regular blood transfusion, despite the fact this therapy is life saver for them, repetition of transfusion provokes the patient's immune system and produce anti-erythrocyte antibodies (Bhatti et al., 2004) a condition known as alloimmunization. Red blood cells alloimmunization results from disparity of antigens between donor and recipient. The production of antibodies can result in clinical haemolysis and cause difficulty in cross matching the blood. In addition, alloimmunization against red blood cell antigens increase the requirement for transfusion and be able to significantly complicate the transfusion therapy.

Alloimmunization to red cell antigens is an immune response is stimulated by the transfusion of blood products and is one of the complications of RBC transfusion dependent patients. Study by Ameen et al. (2003) among multi-transfused thalassaemia patients showed that 30.0% has alloantibody. In addition, study by Hassan et al. (2004) carried out among thalassaemia patient in Islamabad reported that 22.7% of the respondent develops alloantibodies.

In one of the studies the results indicated that the frequency of alloimmunization in thalassaemia patients in northeast Iran is 2.87%. This frequency has been reported in 30% of 190 thalassaemia patients in Kuwait and 4.97% of 161 in Indian patients (Ameen et al., 2003). This may be due to the selection of thalassaemia patients who all had the severe form of the disease (major thalassaemia or intermediate form). The prevalence of alloimmunization in our multi-transfused thalassaemia patients is 18%. This rate is on the low side of the wide range of reported in the other studies. The highest is in the UK at 76 %, Taiwan 37%, Kuwait 30 % and Greece 21.1 % (Ameen et al., 2003; Oljhungbe, 2001).

In previous studies among thalassaemia patient's, the majority of them had received up to 100 units of blood and some of them even received up to 300 units of blood. Much more units of packed red cell transfused to thalassaemia patients compare to others. The total number of transfusions is expected to have an effect on the frequency of alloimmunization (Hassan et al., 2004). Although the relation between the number of blood units transfused and antibody formation is unknown but

still it is an important factor for increased alloimmunization (Ansari et al., 2008). However in our study no relation between those factors can be observed in patients that have received up to 100 units of blood. Among patients who developed alloantibodies there was considerable variation in number of donor exposure required to trigger further development of alloantibodies. In the previous studies one patients develop 5 antibodies after only 21 donor exposure whereas another patient develop anti-K after 46 exposure and anti-Le<sup>a</sup> after 35 more exposures (Vichinsky, 1990). However in the earlier studies no significant difference was found between the rates of alloimmunization of patients SCD patents that transfused more than 10 units of blood. Moreover in the previous studies also noted a much higher total number of transfusions in patients who did not developed alloantibodies. In this studies found that there was no significant difference between the total number of packed cell transfused with the developed of alloantibodies. There were 5 alloantibodies or unexpected antibodies detected in this study. They include anti anti- E, anti-e, anti-c, anti-Jk<sup>b</sup>, and anti Fy<sup>b</sup>. Anti- E detected in 17 patients, anti- e detected in 2 patients, anti-c detected in 4 patients, anti-Jk<sup>b</sup> detected in 2 patients and anti-Fy<sup>b</sup> detected in 1 patient only. Among clinically significant antibodies there were Rh specific (anti-E, anti-e, and anti-c) and Kidd specific (Jk<sup>b</sup>). As expected, patients with multiple antibodies, fortunately were very few in number, do create great problems for the blood bank and some of them have experienced difficulties with cross matching. The pattern of type or specificity of antibody detected in this study is similar to a previous study among multi-transfused patients. Rh related antibodies showed the highest percentage in all studies. In our study 86.67% of the antibodies were detected among Rhesus system. In previous study reported the high prevalence of antibodies (47.7%) that were within the Rh system (Karimi et al., 2007). Moreover Sadeghian et al. (2009) reported 100% of antibodies present against Rhesus blood group (D, c, E) antigens.

Furthermore it was also found that Rhesus group antibodies such as anti-c and anti-E, as well as anti-Kell also reported to be detected in the same population (Ansari et al., 2008). However the other study by Singer (Singer et al., 2000) identified anti-c, anti-E, anti-Kell, anti Jk<sup>b</sup>, anti-Le<sup>a</sup>, anti-M and anti-i. The Rh system antibodies are important in transfusion medicine because these antibodies can cause haemolytic transfusion reactions (Beadling and Cooling, 2007). These antibodies are largely IgG. Immunogenicity of different Rh antigen follows this order: D>c>E>C>e. This study showed that the majority of subjects have single rather than multiple antibodies. Anti-E was the most common alloantibody found which may be determined genetically. The anti-E was detected in approximately all existing studies at relatively high level. In Karimi et al. (2007) reported high

prevalence of antibodies (47.7%) that were reported against Rh system.

The other type of antibodies detected in this study was anti-Jk<sup>b</sup> which belongs to Kidd blood group system. In a study by Bhati et al, 2004 the RBC alloantibodies belonged mainly to Rh system although other such as anti-K, anti-Js<sup>b</sup> anti Jk<sup>a</sup> were also detected. Anti-Jk<sup>a</sup> and anti-Jk<sup>b</sup> are dangerous antibodies because they can be difficult to detect in routine blood cross-matches (Hamilton,2009). They are a common cause of delayed haemolytic transfusion reactions (DHTRs) and are responsible for at least 1/3 of all causes DHTRs. Titration process will decrease the antibody level. It depends on the length of time, since stimulation increase and this may cause antibody become undetectable in patient's blood during current antibody screen. Consequently compatible antigen positive units may in advertently transfuse to patient who developed the antibody. This generally followed by a swift increase in antibody titer and result in DHTR (Hamilton, 2009). Anti -Fy<sup>b</sup> has been identified in 1 patient in this study. Anti-Fy<sup>b</sup> belongs to Duffy system. The duffy system has large number of identified antigens but only 3 antibodies are common which Fy<sup>a</sup>, Fy<sup>b</sup>, Fy3 and Fy5. Antibodies against the Duffy antigens Fy<sup>a</sup>, Fy<sup>b</sup>, Fy3, and Fy5 have all been implicated as the cause of a transfusion reaction. Anti-Fy<sup>a</sup> is more commonly found in patients who are of African descent and have sickle cell anemia and therefore may require multiple blood transfusions (Kim et al., 2004). The patients with multiple antibodies create complications for the Blood Bank as some of them are almost impossible to cross matching (Oljohungbe et al., 2001). This point is of special significance when the haemoglobin concentration drops and need to transfuse become urgent especially where there is severe infection or need for surgery. Chronically transfused patients especially those with multiple antibodies, may require complex, lengthy labour intensive procedure to resolve their serologic problems (Kitchen et al., 1993).

## 5. Conclusion

In conclusion the occurring of alloimmunization in this study the prevalence of alloimmunization was 18%. The type of alloantibody was detected anti-E, anti-e, anti-c, anti-Jk<sup>b</sup> and anti-Fy<sup>b</sup>. There was association can be observed between antibody formation with gender, race, and type of blood group. Furthermore there is no association between formations of antibodies with number of packed red cell transfused. The data showed that alloimmunization to the gender and type of blood group finding in thalassaemia patients and the cause were not fully understood but the effect of multiple allogeneic blood transfusions. Since the most common encountered antibodies being directed against Rh blood group system as can be observed and supported by previous other studies. The extended matching of packed red cells for other

minor antigen especially Rh related antigen should be considered by blood bank service seeing that it can be very helpful in minimizing the rate of alloantibody formation significantly. Furthermore the antibody screening should be performed for Rh (D, C, E) antigens as the most common alloantibodies in all of the patients from the start transfusion to reduce complications. Furthermore in order to reduce the rate of alloantibody synthesis, transfusion of matched packed RBCs for minor blood group especially for Rh (C, E) should be considered in addition to major blood group from the start of transfusion.

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