

Risk Factors Alloimmunization in Children with Transfusion Dependent Thalassemia

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Abstract

Background: Pediatric transfusion-dependent thalassemia (TDT) patients may be at risk of alloimmunization because they require regular blood transfusions throughout their lives. Exposure to repeated blood transfusions can lead to the formation of alloantibodies. Alloimmunization is the formation of alloantibodies against the blood donor recipient, which may cause unwanted transfusion reactions. Alloimmunization can hinder pediatric TDT patients from finding a suitable blood donor, thereby increasing the risk of complications from additional blood transfusion.

Methods: The study employed cross-sectional design with observational analytics conducted at the Polyclinic of Thalassemia Center, Dr. Zainoel Abidin Public Hospital (RSUDZA), Banda Aceh, Aceh. The period was from May 15th to June 24th, 2023, and involved 70 samples that met the research criteria. The data was obtained from a questionnaire administered to the subjects. Blood transfusion and alloantibody screening data were also from the pre-transfusion blood crossmatch examination. Data was analyzed using a bivariate data test performed with a chi-square test.

Results: The results revealed there are 11.43% of patients with alloimmunization in pediatric TDT in Aceh. The bivariate analysis revealed that the most influential risk factor was the volume of blood transfusion ($p=0.009$, $OR=11.083$, 95% CI [1.282-95.786]). The relationship between the duration of blood transfusion ($OR=2.644$) and age 10-8 years ($OR=1.467$) showed no statistically significant influence.

Conclusion: Volume of blood transfusion is the most influential risk factor for alloimmunization in pediatric TDT patients.

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INTRODUCTION

Transfusion Dependent Thalassemia (TDT) is a form of genetic hemoglobin (Hb) disorder characterized by the reduced or absent synthesis of one or more globin chains, resulting in an imbalance in the number of globin chains formed. Regular red blood cell transfusions can reduce the complications of anemia and ineffective erythropoiesis, aid growth and development, and prolong survival in thalassemia major.¹ WHO reported that 40,000 infants are born with a diagnosis of thalassemia- β each year,² with 80% of them coming from middle- to low-income countries. About 0.1% to 0.2% of children with Hb disorders die from thalassemia major.³

Alloimmunization refers to a gradual immunological response triggered by blood transfusions, characterized by the production of antibodies (Ab) against encountered red blood cell antigens (Ag). This phenomenon commonly manifests in pediatric patients with transfusion-dependent thalassemia (TDT), particularly those undergoing repeated transfusions. Alloimmunization can significantly diminish the survival of transfused red blood cells and lead to various complications. These include discrepancies in crossmatch examinations, diminished availability of compatible blood products, necessitation of additional personnel and resources for sourcing matched blood units, accelerated clearance of donor erythrocytes, and the potential for hemolytic transfusion reactions.^{4,5}

Alloimmunization accounts for 10 to 38% of the global incidence of TDT.⁶ Singger, et al found that around 22% of 75% Asian cases developed into alloimmunization.⁷ Alloimmunization research conducted by Kresnawati et al at the Thalassemia Center of Dr. Cipto Mangunkusumo Public Hospital (RSCM) in Jakarta from 2005 to 2013 found that 19% of subjects had alloantibodies.⁸ Furthermore, research in countries around Asia Tamai et al, such as Japan, from 2001 to 2015 and published in 2020, showed a 1.95% positive incidence of alloimmunization in 2,162 samples aged 5 to 15 years.⁹ This study aims to identify the risk factors associated with the occurrence of alloimmunization in pediatric TDT.

METHODS

The study employed a cross-sectional observational analytic design to identify risk factors associated with alloimmunization incidence in children with transfusion-dependent thalassemia (TDT) at dr. Zainoel Abidin Public Hospital (RSUDZA) in Banda Aceh, Indonesia. Sampling was conducted at the Thalassemia Center Polyclinic of RSUDZA Banda Aceh from May 15, 2023, to June 24, 2023, resulting in a sample size of 70 participants. Inclusion criteria comprised children diagnosed with TDT (major/intermedia) by a doctor in the division of the pediatric hematology oncology division at RSUDZA, who had received Packed Red Cells (PRC) transfusions at least five times, with transfusion intervals ranging from two to five weeks, aged between 5 and 18 years, meeting blood transfusion indications, and willing to participate. Exclusion criteria included children diagnosed with non-transfusion-dependent thalassemia (NTDT) (minor/trait), ascertained by a doctor at the pediatric hematology-oncology division at RSUDZA Banda Aceh, those who had received fewer than 5 PRC transfusions, aged below 5 years, and failing to meet blood transfusion requirements or declined to participate.

Questionnaires about TDT were distributed and pre-transfusion blood crossmatch data were collected. Data collection comprised of age, gender, nutritional status, blood type, duration of blood transfusion, and transfusion volume (in cc/kg/month). Alloimmunization screening data and Antibody identification were performed using the Grifols® RBC 11 Identisera Diana panel (1-11). The 70 research subjects were divided into two groups: 8 TDT patients with alloimmunization and 62 TDT patients without alloimmunization. Data were described using tables. Ethical approval was obtained from the Health Research Ethics Committee (KEPK) RSUDZA and the Faculty of Medicine, Syiah Kuala University (No: 066/ETIK-RSUDZA/2023). The bivariate analysis employed the chi-square test using SPSS 26.0 (with a 95% confidence level and a significance level of $p < 0.05$).

Table 1. Alloantibody identification

Case No.	Alloantibody identification type	Frequency
1.	Anti k+ Fy ^a +Fy +Le +S+Co ^b	6
2.	Anti E	1
3	Anti c+K+Jk +P +N ^b ₁	5
4.	Anti K + Jk ^a	2
5.	Anti-Jk ^a +Co ^b	2
6.	Anti C ^w + Kp ^a	2
7.	Anti E+S+ Lu ^a +Xg ^a	4
8.	Anti C+C ^w	2
Total Alloantibodies		24

RESULTS

This study found alloimmunization in pediatric TDT in 8 out of 70 children (11.43%). Half of them (50%) had two alloantibodies, 12.5% had six alloantibodies, 12.5% had five alloantibodies, 12.5% had four alloantibodies, and 12.5% had one alloantibody, as presented in **Table 1**. The distribution and specificity of alloantibodies in all patients are presented in **Table 2**.

Children who met the study criteria demonstrated that, on average, most pediatric patients experiencing Transfusion-Dependent Thalassemia (TDT) were female (54.3%). The mean age of children affected by TDT ranged from 10 to 18 (70%). Moreover, these children categorized based on their nutritional status, with 51.4% classified as having good nutritional status and 48.6% experiencing malnutrition. Blood type analysis revealed the following distribution: blood

type O (40%), blood type B (25.7%), blood type A (22.9%), and blood type AB (11.4%). Furthermore, 74.3% of children with TDT had undergone blood transfusions for over 5 years, with transfusion volumes categorized as <30cc/kg/ml in 39 cases (55.7%) and >30cc/kg/ml in 31 cases (44.3%), as depicted in **Table 3**.

In the bivariate analysis, the variable of blood transfusion volume emerged as a statistically significant risk factor ($p=0.009$, OR=11.053, 95% CI [1.282-95.786]). While no statistical significance was observed in other variables, a correlation was noted between the frequency of blood transfusion therapy exceeding 5 years and the incidence of alloimmunization in pediatric TDT (OR=2.644). Similarly, an association between the age group of 10-18 years and the occurrence of alloimmunization in pediatric TDT patients (OR=1.467), as outlined in **Table 4**.

Table 2. Frequency and Type of Alloantibody Identification

Case No.	Alloantibody Type	Alloimmunization cases 8/70 (11.43%) n (%)
1.	Rh antibody	
	Anti-D	0 (0)
	Anti-C	1 (12,5)
	Anti-E	2 (25)
	Anti-c	1 (12,5)
	Anti-e	0 (0)
	Anti-C ^w	2 (25)
2.	Kell antibody	
	Anti-K	2 (25)
	Anti-k	1 (12.5)
	Anti-Kp ^a	1 (12.5)
	Anti-JS ^a	0 (0)
3	Duffy antibody	
	Anti-Fy ^a	1 (12.5)
	Anti-Fy ^b	1 (12.5)
4.	Kidd antibody	
	Anti-Jk ^a	1 (12.5)
	Anti-Jk ^b	1 (12.5)
5.	Lewis antibody	
	Anti-Le ^a	0 (0)
	Anti-Le ^b	1 (12.5)
6.	P antibodies	
	Anti-P ¹	1 (12.5)
7.	MNS antibody	
	Anti-M	0 (0)
	Anti-N	1 (12.5)
	Anti-S	2 (25)
	Anti-s	0 (0)
8.	Luth antibodies	
	Anti-Lu ^a	1 (12.5)
9	Colt antibody	
	Anti-Co ^b	2 (12.5)
10	Xg antibody	
	Anti-Xg ^a	1 (12.5)
11	Other antibodies	0 (0)
Total Antibody		24

Table 3. Characteristics of research subjects

Characteristics	Alloimmunization Positive n (%)	Alloimmunization Negative n (%)	Total
Gender			
Male	4 (12.5)	28 (87.5)	32 (45.7)
Female	4 (10.5)	34 (89.5)	38 (54.3)
Age			
5-<10 years	3 (14.3)	18 (85.7)	21 (30)
10- 18 years	5 (10.2)	44 (89.8)	49 (70)
Nutrition Status			
Malnutrition	4 (11.8)	30 (88.2)	34 (48.6)
Good nutrition	4 (11.1)	32 (48.4)	36 (51.4)
Duration of transfusion			
< 5 years	1 (5.6)	17 (94.4)	18 (25.7)
> 5 years	7 (13.5)	45 (86.5)	52 (74.3)
Blood Type			
O	4 (14.3)	24 (85.7)	28 (40)
A	0 (0)	16 (100)	16 (22.9)
B	4 (22.2)	14 (77.8)	18 (25.7)
AB	0 (0)	8 (100)	8 (11.4)
Transfusion volume			
< 30 cc/kgb/month	1 (2.6)	38 (97.4)	39 (55.7)
> 30 cc/kgb/month	7 (22.6)	24 (77.4)	31 (44.3)

Table 4. Bivariate analysis of Alloimmunization risk factors in pediatric TDT

Characteristics	Alloimmunization Positive n (%)	Alloimmunization Negative n (%)	P	OR	95% IK
Gender					
Male	4 (12.5)	28 (87.5)	0.796 ^{ct}	1.214	0.278-5.299
Female	4 (10.5)	34 (89.5)			
Age					
5-<10 years	3 (14.3)	18 (85.7)	0.947 ^{ct}	1.467	0.317-6.792
10-18 years	5 (10.2)	44 (89.8)			
Nutrition Status					
Malnutrition	4 (11.8)	30 (88.2)	0.932 ^{ct}	1.067	0.245-4.652
Good nutrition	4 (11.1)	32 (48.4)			
Duration of transfusion					
< 5 years	1 (5.6)	17 (94.4)	0.364 ^{ct}	2.644	0.302-23.122
> 5 years	7 (13.5)	45 (86.5)			
Blood Type					
O	4 (14.3)	24 (85.7)	0.150 ^{kw}	1.093	0.536-2.228
A	0 (0)	16 (100)			
B	4 (22.2)	14 (77.8)			
AB	0 (0)	8 (100)			
Transfusion volume					
<30 cc/kgb/month	1 (2.6)	38 (97.4)	0.009 ^{ct}	11.083	1.282-95.786
>30 cc/kgb/month	7 (22.6)	24 (77.4)			

ct: Chi-square for trend

kw: Kruskal-wallis test

DISCUSSION

The high rate of alloimmunization in Aceh is directly proportional to the high rate of childhood TDT disease in Aceh Province. Previous studies have demonstrated marked variability in the potency of clinically significant blood group antigens, with up to 30-fold differences between some of the highest (e.g., K, E, Jk a) and lowest (e.g., S) immunogenicity antigens¹⁰. Another research by Beshlawy et al.¹¹ studied 200 TDT thalassemia children in Egypt, with the majority of patients (64%) having three alloantibodies, while 28% had two alloantibodies and only 8% had one alloantibody.

Regarding the relationship between age and the incidence of alloimmunization, bivariate tests revealed that the highest incidence was observed in subjects aged >10 years, with around 10% falling into this category, but indicating no statistical significance ($p = 0.947$, OR = 0.947, and 95% CI [0.317-6.792]). This finding aligns with research by Saied et al.¹² on pediatric patients with confirmed alloimmunization in Egypt from 2006 to 2007. Out of 95 thalassemia children studied, those aged ≥ 10 accounted for 32.1% of the cases, compared to children aged ≤ 10 .

The increased risk of alloimmunization in children aged >10 years is attributed to their mature immune system, which can react to allogeneic red blood cell antigens. Conversely, the immature immune system lacks a clear danger signal as each unit of red blood cells is sterile and does not originate from microbes. Moreover, this phenomenon is associated with the unique characteristics of red blood cells, which activate humoral antigens. These antigens possess unique structural features, route of administration, antigen quantity or volume, and duration of exposure, all of which can trigger immune responses in the recipients.¹³

This study found that the most common alloantibody specificities were anti-Rh, anti-Kell, anti-MNS, and anti-Colt. The minor phenotype of anti-Rh included anti-E (25%) and anti-C^w (25%). The most common minor phenotype of anti-Kell was anti-K (25%). The prevalent minor phenotype of anti-MNS was anti-S (25%), while the predominant minor phenotype of anti-Colt was anti-Co^b (25%). Consistent with other

studies conducted by Bhuvu et al.¹⁴ from August 2011 to July 2013 in Gujarat, India, alloantibodies was detected in nine samples from children who received repeated transfusions. The Kell system blood group was attributed to 55% of the alloantibodies, and the Rh system blood group accounted for 4%. Among the five Kell alloantibodies, four were anti-K, and one was anti-Kp^a. Of the four anti-Rh antibodies, three were anti-D, and one was anti-E.

This study examined the relationship between subject characteristics and alloimmunization in TDT patients. This finding contrasts with the study conducted by Zarrabian et al.¹⁵ from July 2018 to June 2022, which involved 92 pediatric TDT patients admitted to Canadian hospitals. In that study, alloimmunization was more prevalent in male patients, with a male-to-female ratio of 3:1. However, there was no statistically significant difference between in alloimmunized and non-alloimmunized patients based on gender ($p=0.796$).

Regarding nutritional status, among children with transfusion-dependent thalassemia (TDT) who test positive for alloimmunization, there were equal proportions of patients with good nutrition and malnutrition (1:1 ratio). Specifically, four cases (11.1%) had good nutrition, and four cases (11.8%) were malnourished ($p=0.932$, OR = 1.067, 95% CI [0.245-4.652]). Statistical analysis did not reveal a significant association between nutritional status and the incidence of alloimmunization in pediatric TDT patients. This finding aligns with a previous study by Harmani et al. (2016)¹⁶, which conducted a cross-sectional investigation at a Tertiary Referral Hospital in Surabaya from October to December 2021. Among the 52 pediatric patients studied, 27 showed compatible alloimmunization, as indicated by Comb's test results. Of these, 14 had good nutrition (51.9%), while 13 were at risk of malnutrition (48.1%). The lower growth rate observed in children with thalassemia often results in a final height as falls short of that of normal children. Consequently, conventional methods for assessing nutritional status, such as upper arm circumference, height-to-weight, age-based anthropometric curves, or Frisancho tables¹⁷, may not

accurately reflect their nutritional status.

The duration of transfusion periods in children with TDT alloimmunization in this study, exceeding 5 years, was observed to be more frequent, with seven cases (13.5%), compared to those with transfusion durations of less than 5 years, where only one case (5.6%) was noted. Despite the lack of statistical significance ($p=0.364$, $OR=2.644$, and 95% CI [0.302-23.122]), this trend is consistent with findings from other studies. For instance, Fridawati et al.'s research¹⁸ at Fatmawati Central General Hospital Jakarta, which involved 81 subjects, identified 10 cases of alloimmunization, with eight cases (9.88%) occurring after transfusion durations exceeding 10 years. Two cases (2.47%) occurred within the 5 to <10 years transfusion period, yielding a statistically significant value ($p=0.008$). These results indicate that alloantibodies tend to emerge after a decade or more of regular blood transfusions

Regarding the association between ABO blood types and TDT alloimmunization in children, our study observed a comparable occurrence in blood types O and B, each represented by four subjects. However, no statistical significance was found ($p=0.150$, $OR=1.093$, and 95% CI [0.536-2.228]). This finding aligns with research by Fateen et al.¹⁹ conducted at Lahore Children's Hospital, Pakistan, from September 2020 to August 2021. In their study, out of 90 cases of TDT children, six cases of alloimmunization were identified, with blood type O accounting for three cases (50%), blood type B for two cases (33.4%), and blood type A for one case (16.6%). All subjects were Rh-positive.

The relationship between the volume of blood transfusions per month and alloimmunization in children with Transfusion-Dependent Thalassemia (TDT) was investigated. It was observed that when the volume of transfusion was <30 cc/kg/month, only one subject showed alloimmunization (2.6%), whereas when the volume exceeded 30 cc/kg/month, seven subjects were alloimmunized (22.6%). This association between transfusion volume and pediatric TDT alloimmunization was statistically significant ($p = 0.009$, $OR= 11.083$, and 95% CI [1.282-95.786]). These findings align

with Yadav et al.'s²⁰ study, conducted prospectively on β -thalassemia patients from December 2021 to September 2022, which reported a higher incidence of alloantibodies in patients receiving greater blood volume per year (240cc/kg/year) compared to those receiving less (155cc/kg/year) ($p < 0.001$). Pathogen exposure history can modulate immune responses to transfused red blood cells, potentially increasing sensitivity to subsequent exposure to antigenically similar red blood cells at the T-cell level.

According to Hendrickson et al.²¹, one of the contributing factors to red blood cell alloimmunization is the abundance and structural diversity of blood group antigens (Ag). Each unit of transfused blood carries numerous Ag, some of which may differ slightly from the recipient's antigens (e.g., K vs k). In contrast, others may be entirely foreign (e.g., Rhesus D), leading to alloimmunization. The significance of minor or variant antigen status in alloimmunization warrants further investigation. Repeated transfusions can promote the formation of erythrocyte alloantibodies in pediatric TDT patients, with higher transfusion volumes correlating with increased alloimmunization incidence.²²

The lack of statistical significance in our study may be attributed to the limited sample size, the homogeneity of the pediatric TDT patient cohort, which may not represent all pediatric TDT subjects in Aceh, and study time potentially impacting the statistical power of the analysis. There is a need for further investigation with more samples, heterogeneity and, longer study time to obtain more accurate data.

CONCLUSION

The occurrence of alloimmunization in pediatric TDT at RSUDZA is 11.43%, with blood transfusion volume is a risk factor for alloimmunization in pediatric TDT patients. Studies on alloimmunization are seldom, so there is a need for this type of research to be conducted in other city or countries.

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AUTHORS' CONTRIBUTIONS

HNH and SM were involved in conceiving and planning the research, SM performed the data acquisition/collection, SM calculated the experimental data and performed the analysis, HNH drafted the manuscript and designed the figures, SM aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

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