How to Cite:
Harmany, P. P., Andarsini, M. R., Ugrasena, I. D. G., Larasati, M. C. S., & Cahyadi, A. (2022). Risk factors for autoimmune hemolytic anemia in children with transfusion-dependent thalassemia at a tertiary referral hospital: A cross-sectional study. *International Journal of Health Sciences*, *6*(S4), 691–701. https://doi.org/10.53730/ijhs.v6nS4.5632

Risk factors for autoimmune hemolytic anemia in children with transfusion-dependent thalassemia at a tertiary referral hospital: A cross-sectional study

Priscilla Putri Harmany
Department of Child Health, Faculty of Medicine Universitas Airlangga, Surabaya, East Java, Indonesia

Mia Ratwita Andarsini
Department of Child Health, Faculty of Medicine Universitas Airlangga, Surabaya, East Java, Indonesia

I Dewa Gede Ugrasena
Department of Child Health, Faculty of Medicine Universitas Airlangga, Surabaya, East Java, Indonesia

Maria Christina Shanty Larasati
Department of Child Health, Faculty of Medicine Universitas Airlangga, Surabaya, East Java, Indonesia

Andi Cahyadi
Department of Child Health, Faculty of Medicine Universitas Airlangga, Surabaya, East Java, Indonesia

***Abstract*---**Repeated transfusions of children with transfusion-dependent thalassemia (TDT) save their lives while causing complications such as the development of autoimmune hemolytic anemia (AIHA). Studies regarding this matter have not yet been extensively reviewed. Therefore, we aim to assess the risk factors of developing AIHA in children with TDT.

***Keywords*---**transfusion-dependent thalassemia, autoimmune hemolytic anemia, risk factors, children, anemia.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
***Corresponding author**: Mia Ratwita Andarsini; Email: mia_r_a@fk.unair.ac.id*
Manuscript submitted: 27 Jan 2022, Manuscript revised: 18 Feb 2022, Accepted for publication: 9 March 2022

Introduction

Transfusion-dependent thalassemia (TDT) is a clinical spectrum of thalassemia in which patients require repeated blood transfusions every 2-6 weeks to maintain hemoglobin (Hb) levels between 9 – 11.5 g/dL.[1-3] Repeated transfusions of TDT patients save their lives while causing complications such as the development of alloimmunization. Repeated exposure to donor erythrocytes by blood transfusion is associated with the formation of autoantibodies (autoimmunization) and alloantibodies (alloimmunization) in TDT patients,[4-6] thus making cross-matching for proper blood supply difficult. Alloimmunization has been reported to occur in 450% of thalassemia patients, with rates varying in some parts of the world.[4] Alloimmunization against erythrocytes was found in 5.6%-8.5% in India, 16.32% in Iran, 4.5% in Iraq.[4-6, 8] Meanwhile, autoantibodies against erythrocytes were found in 4.7% of 63 patients with thalassemia in Malaysia, in 1.42%-28.2% in India.[4-6] Factors associated with the incidence of autoimmune hemolytic anemia (AIHA) in TDT patients, including gender, age at first transfusion, type of blood transfusion, transfusion volume, transfusion interval, splenectomy, type of thalassemia, blood type, serum ferritin level, and pre-transfusion Hb level.[7] Transfusion reactions will increase with high hemolysis reactions, due to shortened erythrocyte age, which will increase the need for transfusion. Therefore, blood supply at hospital blood banks or transfusion facilities becomes difficult and restricted. This requires AIHA treatment, which incurs additional costs and length of stay.

The formation of alloimmunization and alloantibodies against erythrocyte antigens lead to difficulties in cross-matching, shortening the in vivo survival of transfused erythrocytes, causing transfusion reactions, difficulty in providing safe transfusions and cross-matching, thus increasing the risk of iron overload.[9] Alloimmunization factors are complex and involve three main elements including differences in red cell antigens between blood donors and recipients, the immune status of the recipient, and the immunomodulatory effect of allogeneic blood transfusion on the immune system of the recipient. [10, 11]Low alloimmunization rates can be expected when there is a homogeneity of RBC antigens between blood providers and recipients.[10] Therefore, it is necessary to know the risk factors for AIHA in children with TDT. Studies that have determined risk factors for AIHA in children with TDT have not yet been extensively reviewed.

Method

A cross-sectional study was conducted in Tertiary Referral Hospital, Surabaya, Indonesia from October to December 2021. The inclusion criteria for this study were 1) 0-18-year-old pediatric patients diagnosed with transfusion-dependent thalassemia at our Pediatric Haemato-Oncology Outpatient Installation during the study period, 2) having received blood transfusions at least 10 transfusion, and 3) A signed informed consent by parents or legal guardians to participate in this study. We excluded patients with comorbid conditions that affect the immune system such as cancer or Systemic lupus erythematosus (SLE), Children who take corticosteroids, and other immunosuppressant drugs such as nephrotic syndrome, allergic diseases, and arthritis, and refuse to be research respondents.

Ethical clearance has been approved by the Clinical Research Unit, Dr. Soetomo General Hospital with number 0287/KEPK/X/2021.

Participants who meet the inclusion criteria will undergo a complete examination including data collection, history taking, physical examination of the patient, and taking blood specimens for examination at the Clinical Pathology Laboratory at our hospital. We examined several risk factors for the incidence of AIHA in children with TDT through the results of the Coomb's test. The incidence of AIHA in children with TDT whose Coomb's test results are incompatible. The risk factors that we studied were age, age at diagnosed, age at first transfusion,[12, 13] transfusion interval,[14, 15] duration of transfusion (years), history of splenectomy, blood type, nutritional status,[16-18] average of Hb per transfusion (g/dL), ferritin level (ug/L), number of blood transfusions per year (mL/kg).[19]

Data analysis

The data is displayed in tabular descriptive statistics. IBM SPSS Statistics Version 25 was used for statistical analysis. We presented frequency and percentage, mean ± standard deviation (SD) if the variables were normally distributed, and the median Interquartile range (IQR) for variables that were not normally distributed. Normality test using Kolmogorov-Smirnov test. Bivariate and multivariate analyses were used to assess the correlation between the independent variable and the dependent variable using the Pearson chi-square test or Fisher's Exact test and logistic regression. Mann-Whitney test was used to determine the difference between two variables that were not normally distributed. In data analysis, the value of Odds Ratio (OR), 95% confidence interval (CI) will be obtained. The results of the study were considered significant if the p-value <0.05.

Results

Participants in this study involved 52 pediatric patients with TDT who met the inclusion and exclusion requirements. All pediatric patients were diagnosed with TDT. Table 1 shows most of the data on the clinical characteristics of children with TDT. Several risk factors that have a correlation with the incidence of AIHA in children with TDT include the child's age, age at first receiving a transfusion, transfusion interval, and duration of transfusion ($p = 0.009$, $p = 0.013$, $p = 0.020$, $p = 0.012$). (Table 2). There was a significant difference between the age at diagnosis in children with thalassemia depending on the incidence of autoimmune hemolytic anemia with a p-value of 0.004.

Table 3 shows the results of the bivariate test in children with TDT on the incidence of AIHA. There are five variables that have a significant effect on the incidence of AIHA, including age, age at diagnosis, age at first receiving a transfusion, transfusion interval, and the duration of transfusion. Based on the multivariate analysis in Table 4, shows that the variables that have a significant influence on the incidence of AIHA are age at first transfusion and transfusion interval with a p-value of 0.004, 0.021 respectively. In this analysis, it was found that children with TDT who received their first transfusion were under three years old and had a 13.4 times greater risk of developing AIHA compared to those who had their first transfusion aged ≥3 years old. It should be noted that children with

TDT who had a transfusion interval of less than 3 weeks had a 6.4 times greater risk than those with a transfusion interval ≥3 weeks.

Discussion

This study found a fairly high prevalence, which was about 27 of 52 (51.9%) children with TDT experiencing AIHA. Another study in Egypt by El-Beslawy showed 36 of 200 (18%) patients were alloimmunized, a study by Abdelrazik showed 15 of 188 (7.98%) patients were alloimmunized and a study by Saied showed 27 (28.4%) of 95 patients had alloimmunization.[20-22] The overall prevalence of RBC alloimmunization among registrants in the Thalassemia Clinical Research Network (TCRN) registry and The Thalassemia Longitudinal Cohort (TLC) was 16.6%, twice the rate in patients with hematological malignancies, and greater than the clinically significant antibody prevalence reported for the general population. The impact of antibody detection on transfusion leads to the cessation of transfusion or an increase in the need for transfusion. In this study, we found a higher incidence of autoantibodies, i.e. the results of the Coombs test were compatible in 25 subjects (48.1%) and incompatible in 27 subjects (51.9%). Nearly 52% of patients involved in this study were male. Thalassemia is autosomal dominant; men and women have the same risk of thalassemia.[23, 24]

Patients with β-thalassemia major require regular blood transfusions, supported by appropriate iron chelation therapy (ICT), throughout their lives. β-thalassemia major is a global disease with the highest prevalence in Southeast Asia, Africa, and the Mediterranean.[25] This study involved 52 children with TDT with the type of β-thalassemia major who had an average age at diagnosis of 131.65 months or more than 12 years. The Coomb's test is considered a diagnostic feature of AIHA. AIHA can occur due to the formation of RBC autoantibodies and alloimmunization. Singer et al found a high frequency (25%) of red cell autoantibody formation in 64 thalassemia patients of Asian descent, mostly warm IgG antibodies, of which 18% had clinically significant hemolysis.[10, 26]

Up to 15% of children with TDT had a history of splenectomy, but the clinical factors of splenectomy did not have a strong impact on the development of AIHA. Other studies have been consistent in identifying alloantibodies, transfusion exposure, and splenectomy as risk factors for the development of autoantibodies. Interestingly, only two observational studies and a few case reports evaluating AIHA are clinically relevant, with prevalence ranging from 1.8% to 6.4%.[7, 27] Motta et al. also showed that splenectomy in children with a positive direct antiglobulin (Coomb's test) increased the risk of AIHA in patients with thalassemia.[28] Contrary to the previous statement, the results of this study found that patients with a history of splenectomy were less likely to develop AIHA events compared to patients without a history of splenectomy based on this study. Several other studies have also obtained similar results.[8, 9, 22, 29-31]

Transfusion interval showed a significant effect on the incidence of AIHA in bivariate and multivariate analysis. Longer transfusion intervals have a greater tendency to develop AIHA compared to smaller transfusion intervals. However, a study showed that the transfusion interval did not affect on the incidence of

alloimmunization.[22] The Moroccan study also reported a significant correlation between transfusion interval and alloantibody formation.[14]
Transfusion duration of more than 10 years is a significant risk factor for alloimmunization. Previous studies have shown that, compared with patients who have been transfused regularly for 10 years or less, patients who have been transfused for 20 years or more are more likely to have alloimmunization.[32] A study at Hasan Sadikin Hospital Bandung, obtained positive alloantibody screening results in 2 study subjects (1.1%) with transfusion duration >10 years.[33] Our study shows that blood type did not significantly influence the incidence of AIHA in this study. These results are consistent with several previous studies.[20, 22, 29-31] Another study has shown that blood type AB can predict the incidence of AIHA. However, the type of leukoreduce transfusion has a protective effect against AIHA so this may explain the results of this study.[7]

We found no significant association between nutritional status as a risk factor for AIHA. Nutrition can affect the work of the body's immune system. Theoretically, excess nutrition can increase the activity of the human immune system, while insufficient nutrition can suppress the work of immune cells.[34] It is known that the immune system is affected by dietary factors and that malnutrition can lead to immunodeficiency. Even a deficiency of a single nutrient can lead to altered immune responses in the body. The Hb level before transfusion did not significantly influence the incidence of autoimmune hemolytic anemia based on the results of this study. These results are similar to some previous studies.[8, 35] However, a study in Italia stated that anemia severity was identified as the strongest predictor of recurrence of AIHA, and Hb < 8 g/dL at onset had a 4-fold increased risk of recurrence.[36] The variety of results obtained by different researchers included differences ages at the start of transfusions, differences number of patients studied, differences duration of each study, and similarity of erythrocyte antigens between recipients and donors in each region.

Research on risk factors for AIHA, especially in children with TDT, is still rare. However, we believe this study can be useful for health workers to pay more attention to some supporting clinical factors in the management of thalassemia in children including the transfusion interval, how long the patient underwent transfusion therapy and the age at first receiving a transfusion. In conducting this research, some improvements need to be made in the future to obtain better research results, such as increasing the variation in the number of research subjects, measuring several other clinical supporting factors such as autoantibody and alloantibody data, family history of autoimmune hemolytic anemia, history of autoantibodies and recipient plasma alloantibodies, and other supportive therapies.

Discussion

Results should be clear and concise. Discussion should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature (Berg et al., 2004), the Results section reports what was found in the study, and the Discussions section explains the meaning and significance of the results and provides suggestions for future directions of research. In this section,

it is explained the results of the research and at the same time is given a comprehensive discussion. Results can be presented in figures, graphs, tables, and others that make the reader understand easily. The discussion can be made in several sub-chapters.

Table 1. Clinical characteristics of research subjects

Clinical characteristics N = 52	Value
Sex	
Male	27 (51.9)
Female	25 (48.1)
Age (years old)	
4 – 6	15 (28.8)
7 - 12	16 (30.8)
13 - 18	21 (40.4)
Age at diagnosed (months)	131.65 ± 49.68
	132 [87 - 177]
Age at first transfusion (years old)	
< 3	30 (57.7)
≥ 3	22 (42.3)
Number of blood transfusions per year (mL/kg)	
80 – 119	25 (48.1)
120 – 159	10 (19.2)
160 – 199	10 (19.2)
200 – 240	3 (5.8)
>250	4 (7.7)
Transfusion interval (weeks)	
< 3	21 (40.4)
≥ 3	31 (59.6)
History of splenectomy	
Yes	8 (15.4)
No	44 (84.6)
Blood type	
A	7 (13.5)
B	16 (30.8)
O	4 (7.7)
AB	25 (48.1)
Nutritional status	
Normal	27 (51.9)
Potential malnutrition	24 (46.2)
Severe malnutrition	1 (1.9)
Average of Hb per transfusion (g/dL)	
< 9	51 (95.1)
≥ 9	1 (1.9)
Ferritin levels (ug/L)	
< 2500	20 (38.5)
≥ 2500	32 (61.5)
Duration of transfusion (years)	

Clinical characteristics N = 52	Value
< 10	20 (38.5)
≥ 10	32 (61.5)
Coomb's test	
Compatible	25 (48.1)
Incompatible	27 (51.9)

Data was presented as N(%), Mean ± Standard Deviation, Median [Interquartile Range]

Table 2. Correlation of clinical risk factors for AIHA in children with TDT

Risk factors	Coomb's test		p-value
	Compatible N = 25	Incompatible N = 27	
Sex			0.991[a]
Male	13 (52)	14 (51.9)	
Female	12 (48)	13 (48.1)	
Age (years old)			0.009[b]*
4 – 6	11 (44)	4 (14.8)	
7 - 12	8 (32)	8 (29.6)	
13 - 18	6 (24)	15 (55.6)	
Age at diagnosed (months)	96 [60 - 156]	156 [132 - 180]	0.004[c]*
Age at first transfusion (years old)			0.013*
< 3	10 (40)	20 (74.1)	
≥ 3	15 (25)	7 (25.9)	
Number of blood transfusions per year (mL/kg)			0.575[a]
80 – 119	13 (52)	12 (44.4)	
120 – 159	5 (20)	5 (18.5)	
160 – 199	3 (12)	7 (25.9)	
200 – 240	1 (4)	2 (7.4)	
>250	3 (12)	1 (3.7)	
Transfusion interval (weeks)			0.020 [a]*
< 3	6 (24)	15 (55.6)	
≥ 3	19 (76)	12 (44.4)	
History of splenectomy			0.906[a]
Yes	4 (16)	4 (14.8)	
No	21 (84)	23 (85.2)	
Blood type			0.522[a]
A	5 (20)	2 (7.4)	0.184[a]
B	6 (24)	10 (37)	0.309[a]
O	2 (8)	2 (7.4)	0.936[a]
AB	12 (48)	13 (48.2)	0.991[a]
Nutritional status			0.569[a]
Normal	13 (52)	14 (51.9)	
Potential malnutrition	11 (44)	13 (48.1)	
Severe malnutrition	1 (4)	0 (0)	
Average of Hb per transfusion (g/dL)			0.294[a]
< 9	24 (96)	27 (100)	

≥ 9	1 (4)	0 (0)	
Ferritin levels (ug/L)			0.430[a]
< 2500	11 (44)	9 (33.3)	
≥ 2500	14 (56)	18 (66.7)	
Duration of transfusion (years)			0.012[a]*
< 10	14 (56)	6 (22.2)	
≥ 10	11 (44)	21 (77.8)	

[a]Pearson Chi-Square Test; *p-value < 0.05 was significant; [b]Fisher Exact test; [c]Mann Whitney U Test; Median [IQR]

Table 3. Bivariate analysis of risk factors influencing the incidence of AIHA in children with TDT

Risk factors	p-value	OR	95% CI (lower – upper)
Age (years old)	0.011*	0.145	0.033 – 0.642
4 – 6			
7 - 12			
13 - 18			
Age at diagnosed (months)	0.004*	1.020	(1.006 – 1.034)
Age at first transfusion (years old)	0.015*	4.286	1.323 – 13.881
< 3			
≥ 3			
Transfusion interval (weeks)	0.024*	3.958	1.203 – 13.025
< 3			
≥ 3			
Duration of transfusion (years)	0.015*	0.224	0.067 – 0.747
< 10			
≥ 10			

Binary Logistic Regression; *p-value < 0.05 was significant; Odd Ratio (OR); Confidence Interval (CI)

Table 4. Multivariate analysis of risk factors that influence the incidence of AIHA in children with TDT

Risk factors	p-value	OR	95% CI (lower – upper)
Age (years old)	0.092	0.153	0.017 – 1.362
4 – 6			
7 - 12			
13 - 18			
Age at diagnosed (months)	0.869	1.002	0.997 – 1.028
Age at first transfusion (years old)	0.004*	13.489	2.257 – 80.628
< 3			
≥ 3			
Transfusion interval (weeks)	0.021*	6.470	1.327 – 31.540
< 3			
≥ 3			
Duration of transfusion (years)	0.083	0.177	0.025 – 1.257
< 10			
≥ 10			

Binary Logistic Regression; *p-value < 0.05 was significant; Odd Ratio (OR); Confidence Interval (CI)

Conclusion

A study at a tertiary referral hospital showed that the age at the first transfusion and the transfusion interval was the most influential risk factors for developing AIHA in children with TDT. We hope that these results may encourage the pediatricians in calculating the risk of AIHA in children with TDT and considering appropriate treatments for management of TDT in children. However, further research is needed to consider other contributing factors such as autoantibody and alloantibody data, family history of autoimmune hemolytic anemia, history of recipient plasma autoantibodies, and alloantibodies, and other supportive therapies.

References

Permono B, Sutaryo, S., Ugrasena, IDG., Windiastuti, E. *Buku Ajar Hematologi-Onkologi Anak.* Jakarta: Badan Penerbit IDAI., 2012.

Sari TT, Gatot D, Akib AAP, et al. Immune response of thalassemia major patients in Indonesia with and without splenectomy. *Acta Medica Indonesiana* 2016; 46.

Wahidiyat I. Thalassemia dan permasalahannya di Indonesia. *Sari Pediatri* 2016; 5: 2-3.

Datta SS, Mukherjee S, Talukder B, et al. Frequency of red cell alloimmunization and autoimmunization in thalassemia patients: a report from Eastern India. *Advances in hematology* 2015; 2015.

Dhawan HK, Kumawat V, Marwaha N, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. *Asian journal of transfusion science* 2014; 8: 84.

Dogra A, Sidhu M, Kapoor R, et al. Study of red blood cell alloimmunization in multitransfused thalassemic children of Jammu region. *Asian Journal of Transfusion Science* 2015; 9: 78.

Khaled MB, Ouederni M, Sahli N, et al. Predictors of autoimmune hemolytic anemia in beta-thalassemia patients with underlying red blood cells autoantibodies. *Blood Cells, Molecules, and Diseases* 2019; 79: 102342.

Al-Mousawi MMN, Al-Allawi NAS and Alnaqshabandi R. Predictors of red cell alloimmunization in Kurdish multi transfused patients with hemoglobinopathies in Iraq. *Hemoglobin* 2015; 39: 423-426.

Karimi M, Nikrooz P, Kashef S, et al. RBC alloimmunization in blood transfusion-dependent β-thalassemia patients in southern Iran. *International journal of laboratory hematology* 2007; 29: 321-326.

Singer ST, Wu V, Mignacca R, et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood, The Journal of the American Society of Hematology* 2000; 96: 3369-3373.

Ahmed Kiani R, Anwar M, Waheed U, et al. Epidemiology of transfusion transmitted infection among patients with β-thalassaemia major in Pakistan. *Journal of blood transfusion* 2016; 2016.

Michail-Merianou V, Pamphili-Panousopoulou L, Piperi-Lowes L, et al. Alloimmunization to red cell antigens in thalassemia: comparative study of usual versus better-match transfusion programmes. *Vox sanguinis* 1987; 52: 95-98.

Spanos TH, Karageorga M, Ladis V, et al. Red cell alloantibodies in patients with thalassemia. *Vox sanguinis* 1990; 58: 50-55.

El Kababi S, Benajiba M, El Khalfi B, et al. Red blood cell alloimmunizations in beta-thalassemia patients in Casablanca/Morocco: Prevalence and risk factors. *Transfusion Clinique et Biologique* 2019; 26: 240-248.

Koçyiğit C, Eliaçık K, Kanık A, et al. Frequency of red cell allo-and autoimmunization in patients with transfusion-dependent beta thalassemia and affecting factors. *Turk J Pediatr* 2014; 56: 487-492.

Kuczmarski RJ. *2000 CDC Growth Charts for the United States: methods and development*. Department of Health and Human Services, Centers for Disease Control and …, 2002.

Merritt RJ and Suskind RM. Nutritional survey of hospitalized pediatric patients. *The American Journal of Clinical Nutrition* 1979; 32: 1320-1325.

World Health O. *WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development*. World Health Organization, 2006.

Cappellini M-D, Cohen A, Porter J, et al. *Guidelines for the management of transfusion dependent thalassaemia (TDT)*. Thalassaemia International Federation Nicosia, Cyprus, 2014.

Abdelrazik AM, Elshafie SM, El Said MN, et al. Study of red blood cell alloimmunization risk factors in multiply transfused thalassemia patients: role in improving thalassemia transfusion practice in Fayoum, Egypt. *Transfusion* 2016; 56: 2303-2307.

El-Beshlawy A, Salama AA, El-Masry MR, et al. A study of red blood cell alloimmunization and autoimmunization among 200 multitransfused Egyptian β thalassemia patients. *Scientific Reports* 2020; 10: 1-8.

Saied DA, Kaddah AM, Eldin RMB, et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent Egyptian thalassemic patients. *Journal of pediatric hematology/oncology* 2011; 33: 409-414.

Fucharoen S and Weatherall DJ. Progress toward the control and management of the thalassemias. *Hematology/oncology Clinics* 2016; 30: 359-371.

Weatherall D. The thalassemias: the role of molecular genetics in an evolving global health problem. *The American Journal of Human Genetics* 2004; 74: 385-392.

Shah FT, Sayani F, Trompeter S, et al. Challenges of blood transfusions in β-thalassemia. *Blood reviews* 2019; 37: 100588.

Xu L-H, Fang J-P, Weng W-J, et al. Autoimmune hemolytic anemia in patients with β-thalassemia major. *Pediatric Hematology and Oncology* 2012; 29: 235-240.

Aygun B, Padmanabhan S, Paley C, et al. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. *Transfusion* 2002; 42: 37-43.

Motta I, Giannotta J, Ferraresi M, et al. Autoimmune Hemolytic Anemia as a Complication of Congenital Anemias. A Case Series and Review of the Literature. *Journal of Clinical Medicine* 2021; 10: 3439.

Abdulqader AMR, Mohammed AI and Mohammed NI. Red cell alloimmunization and autoimmunization in multi-transfused thalassemia patients in Sulaymaniyah Province-Iraq. *Korean Journal of Clinical Laboratory Science* 2020; 52: 98-104.

Al-Riyami AZ, Al-Muqbali A, Al-Sudiri S, et al. Risks of red blood cell alloimmunization in transfusion-dependent β-thalassemia in Oman: a 25-year experience of a university tertiary care reference center and a literature review. *Transfusion* 2018; 58: 871-878.

Waheed U, Arshad M, Saeed M, et al. Spectrum of alloimmunization among multitransfused beta-thalassemia major patients. *Global Journal of Transfusion Medicine* 2019; 4: 39.

Thompson AA, Cunningham MJ, Singer ST, et al. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. *British journal of haematology* 2011; 153: 121-128.

Perwitasari E, Dalimoenthe NZ, Lismayanti L, et al. Alloantibody Screening on Transfusion Dependent Thalassemia Patients in Dr. Hasan Sadikin General Hospital, Bandung. *JOURNAL OF THE INDONESIAN MEDICAL ASSOCIATION Majalah Kedokteran Indonesia* 2017; 67.

Alwarawrah Y, Kiernan K and MacIver NJ. Changes in nutritional status impact immune cell metabolism and function. *Frontiers in immunology* 2018; 9: 1055.

Jansuwan S, Tangvarasittichai O and Tangvarasittichai S. Alloimmunization to red cells and the association of alloantibodies formation with splenectomy among transfusion-dependent β-thalassemia major/HbE patients. *Indian Journal of Clinical Biochemistry* 2015; 30: 198-203.

Barcellini W, Fattizzo B and Zaninoni A. Management of refractory autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation: current perspectives. *Journal of blood medicine* 2019; 10: 265.