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Original Article

The Hemoglobin Levels and Transfusion Intervals of Beta-Thalassemia Patients with Positive and Negative Allo-Autoantibodies

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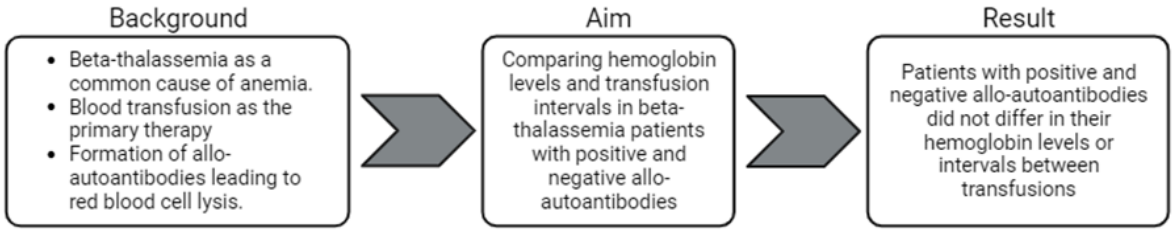
ABSTRACT

Beta-thalassemia is a common cause of anemia. Blood transfusion is the primary therapy for beta-thalassemia patients in Indonesia who are at risk of developing allo-autoantibodies. These antibodies can lead to the lysis of red blood cells, resulting in a rapid decrease in hemoglobin levels and shorter transfusion intervals. This research aims to compare hemoglobin levels and transfusion intervals in beta-thalassemia patients with positive and negative allo-autoantibodies. This research is a retrospective cohort study utilizing medical record data from beta-thalassemia patients who underwent blood transfusions at Dr. Soetomo Hospital. The patients were divided into two groups: those with positive allo-autoantibodies and those with negative allo-autoantibodies. Data on hemoglobin levels and transfusion dates were collected five times consecutively between July and December, 2021. The hemoglobin levels of the two groups were compared using the Mann-Whitney test, while the transfusion intervals were analysed using the T-test. Data were obtained from 52 beta-thalassemia patients who received transfusions, with 25 (48%) testing positive for allo-autoantibodies and 27 (52%) testing negative. It was observed that the hemoglobin levels of the two groups were not significantly different ($p = 0.769$). Similarly, the transfusion intervals of the two groups were not significantly different ($p = 0.899$). There were no significant differences in hemoglobin levels and transfusion intervals between patients with positive and negative allo-autoantibodies.

GRAPHICALABSTRACT



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Introduction

Beta-thalassemia stands out as a prevalent cause of anemia, originating from a diminished or absent synthesis of the beta-globin chains of hemoglobin [1]. It represents the most common inherited single-gene abnormality [2]. Indonesia, being part of the global thalassemia belt, exhibits a high frequency of the thalassemia gene, ranging from 3-10% [3]. Reports from the Eijkman National Molecular Institute indicate a carrier gene frequency of beta-thalassemia in Indonesia within this range [4]. As of 2019, the estimated number of beta-thalassemia patients in Indonesia reached approximately 10,000 [5]. The current approach to managing beta-thalassemia is predominantly symptomatic, involving lifelong blood transfusions [6]. For beta-thalassemia patients, the indication for transfusion is a hemoglobin level below 7 g/dL in two consecutive examinations, with an interval exceeding two weeks and without signs of infection. Alternatively, a hemoglobin level above 7 g/dL may prompt transfusion if there is evidence of failure to thrive or bone deformities resulting from thalassemia [7].

The targeted pre-transfusion hemoglobin level is set at 9-10 g/dL, while post-transfusion levels aim for 13-14 g/dL. The typical transfusion interval for beta-thalassemia patients ranges from 2 to 4 weeks [8]. Repeated blood transfusions in transfusion-dependent beta-thalassemia patients elevate the risk of infectious disease transmission, volume overload, hemolytic transfusion reactions, iron overload, allo- and autoimmunization, and Delayed Haemolytic Transfusion Reactions (DHTRs) [6, 9, 10].

Allo-autoantibodies contribute to the lysis of red blood cells, shortening their lifespan, diminishing pre-transfusion hemoglobin levels, and requiring more frequent transfusion intervals [7, 11]. Kurniawan *et al.*'s research revealed that transfusion-dependent beta-thalassemia patients with allo-autoantibodies failed to maintain hemoglobin levels after transfusion compared to those without allo-autoantibodies. This finding aligns with Essa *et al.*'s research, indicating that transfusion-dependent beta-thalassemia patients with allo-autoantibodies require significantly more frequent transfusions per year [12, 13].

This study aims to compare hemoglobin levels and transfusion intervals in transfusion-dependent beta-thalassemia patients, specifically between groups with positive and negative allo-autoantibodies, at Dr. Soetomo Hospital.

Martials and Methods

This retrospective cohort study was conducted in August-September 2022, with research data obtained subsequent to receiving approval from the Health Research Ethics Committee of Dr. Soetomo Hospital (approval letter number: 1006/LOE/301.4.2/VIII/2022). The study involved the collection of medical record data from beta-thalassemia patients who underwent transfusions at Dr. Soetomo Hospital in Surabaya, East Java, Indonesia. Inclusion criteria comprised patients whose medical records confirmed a diagnosis of beta-thalassemia for minimal six months and who had received a minimum of 5 blood transfusions between July and December 2021. Exclusion criteria encompassed beta-thalassemia patients who had received fewer than 5 transfusions, and patients with infectious, malignant, or degenerative diseases based on medical records. Hemoglobin data, based on medical record information, was measured using the Sysmex Automated Hematology Analyzer XN-3000 (Sysmex, Kobe, Japan) utilizing the SLS-Hemoglobin method. Alloantibody data were determined through antibody screening in pretransfusion tests using the QWALYS 3 (Diagast, Loos, France) with the solid-phase adherence method. Autoantibody data were assessed based on auto-control results using Diamed gel cards (Bio-Rad, Cressier, Switzerland).The collected data were categorized into two groups: the positive allo-autoantibody group and the negative allo-autoantibody group.

The Kolmogorov-Smirnov test was employed to assess the normality of the research data. Differences in hemoglobin levels between the two groups were analysed using the Mann-Whitney test, while differences in transfusion intervals were analysed using the T-test. The data was processed using SPSS version 25 (IBM Corp.,

United States), with a significance level set at $p < 0.05$.

Results and Discussion

Patient characteristics

A total of 52 data sets from transfusion-dependent beta-thalassemia patients were analyzed in this study, with 25 patients (48%) in the positive allo-autoantibody group and 27 patients (52%) in the negative allo-autoantibody group. Patient characteristics are summarized in Table 1.

The antibody examination of transfusion-dependent beta-thalassemia patients revealed that 10 patients (19.2%) exhibited both allo-autoantibodies. In addition, 21 patients (40%) had autoantibodies, while 14 patients (27%) had alloantibodies. Notably, the number of patients exclusively presenting autoantibodies (n = 11) surpassed those with only alloantibodies (n = 4) (Table 2).

Comparison of hemoglobin levels and transfusion intervals

The comparison of hemoglobin levels and transfusion intervals in transfusion-dependent beta-thalassemia patients between the positive and negative allo-autoantibody groups did not yield significant differences ($p>0.05$), as indicated in Table 3.

Table 1: Patient characteristics

Characteristics	Allo-Auto Antibody Positive n (%)	Allo-Auto Antibody Negative n (%)
Total	25 (48)	27 (52)
Gender		
Man	5 (20)	15 (55.6)
Woman	20 (80)	12 (44.4)
Age		
≤ 18 years	15 (60)	19 (70.4)
> 18 years old	10 (40)	8 (29.6)
Number of transfusions		
≤ 50 times	8 (32)	9 (33.3)
51-150 times	9 (36)	11 (40.7)
151-250 times	5 (20)	7 (26)
251-350 times	3 (12)	0

Table 2: The occurrence of allo-autoantibodies

	Alloantibodies	
	Positive n=14 (27%)	Negative
Autoantibodies		
Positive n=21 (40%)	10	11
Negative	4	27

Table 3: Comparison of hemoglobin levels and transfusion intervals between positive and negative allo-autoantibody groups

Variable	Positive allo-autoantibody	Negative allo-autoantibody	*P*-value
Hemoglobin level (Median)	7.9 g/dL	7.92 g/dL	0.769
Transfusion intervals (Mean)	3.31 weeks	3.27 weeks	0.899

In this study, it was observed that the positive allo- and autoantibody group was predominantly composed of women, aligning with findings from research by Essa *et al.* and Singer *et al.* These studies suggest that nulliparous women are at a higher risk of experiencing Red Blood Cell (RBC) sensitization [13, 14]. However, the contrary results were obtained from the research by Ameen *et al.*, Dhawan *et al.*, Hendrickson *et al.*, and Saifeldeen *et al.*, which indicated no significant relationship between gender and the incidence of allo- and autoantibodies in transfusion-dependent beta-thalassemia patients [15-18]. The age distribution of patients in our study did not show significant differences between the two groups, aligning with Dhawan *et al.*'s research [16]. The relationship between the number of transfusions and the occurrence of allo- and autoantibodies remains unclear, as suggested by the literature [19]. In our study, the average number of transfusions in both groups was identical, at 100.26 times. This finding is consistent with the research by Dhawan *et al.*, Saifeldeen *et al.*, Ahmed *et al.*, and Obeidi *et al.* [16, 18, 20, 21]. In contrast, Singer *et al.* and Vichinsky *et al.* reported that a higher number of transfusions is associated with an increased incidence of allo-autoantibodies [14, 22]. Notably, our study identified three patients with allo- and autoantibodies who had undergone 300 or more transfusions, with ages ranging from 30, 33, to 38 years. This suggests advancements in prevention and management efforts, contributing to an increased life expectancy for transfusion-dependent beta-thalassemia patients in Indonesia compared to the situation in 1978 when life expectancy was around 8-10 years [5].

Alloantibodies are an immune response stimulated by repeated PRC (Packed Red Cell) transfusions. Several factors that can cause the formation of alloantibodies are differences in RBC antigens between donor and recipient blood, the recipient's immune status, and the immunomodulatory effect on the recipient's immune system [14, 16, 23]. Previous studies reported the incidence of alloantibodies varying from 4-50% [14, 16, 23, 24]. High incidence is observed when donor and recipient populations are heterogeneous, compatibility testing procedures are lacking, the age at which transfusion begins is > 1 year, nonleucoreduced PRC is administered, and a history of splenectomy exists [7, 14, 16, 18, 23, 24]. Initiating transfusions after the age of 1 year can lead to alloantibody formation due to the loss of protection from the immature immune system and the breakdown of adaptive immune tolerance to allogeneic RBC antigens [16, 19, 24] Administration of nonleucoreduced PRC and a history of splenectomy can also contribute to alloantibody appearance by inducing lymphocytosis associated with blood transfusions, leading to increased serum immunoglobulins, immune complexes, and cells expressing immunoglobulins [14, 16, 25]. This study found a relatively high incidence of alloantibodies in transfusion-dependent beta-thalassemia patients, specifically at 27%. This aligns closely with the findings of Saifeldeen *et al.* (23.1%) and Singer *et al.* (22%) [14, 18]. Potential contributors to alloantibody formation in our study include population heterogeneity, a suspected age at which transfusion begins exceeding 1 year, and a suspected history of splenectomy.

In this study, 40% of patients were found to have autoantibodies, which is notably higher than reported in previous research by Dhawan *et al.* (28.2%), Saifeldeen *et al.* (9.2%), Singer *et al.* (25%), Ameen *et al.* (11%), Pahuja *et al.* (0.47%), and Noor *et al.* [14-16, 18, 23, 26]. The factors contributing to the formation of autoantibodies in transfusion-dependent beta-thalassemia patients remain poorly understood. Singer *et al.* and Dhawan *et al.* propose a connection between autoantibody formation and a history of splenectomy, suggesting that the loss of an efficient filtration system leads to the exposure of old Red Blood Cells (RBCs) to new antigens, triggering an immune response, including the formation of autoantibodies. However, Noor *et al.* found no association between the occurrence of autoantibodies and a history of splenectomy [14, 16, 26]. Singer *et al.* further reported that the incidence of autoantibodies is related to a history of previous alloantibodies and the administration of nonleucoreduced PRC. In contrast, Noor *et al.* proposed that autoantibody formation can be

influenced by storing PRC at a temperature of 1-6 °C for more than 3 days. This storage condition induces an increase in White Blood Cell (WBC) apoptosis, releasing immunostimulatory antigens and biological mediators (core protein matrix, CTLA-4 epitope), thereby sensitizing the patient's immune system and triggering autoantibody formation [14, 26-29]. The high prevalence of patients with autoantibodies in our study is suspected to be influenced by previous alloantibodies and a history of splenectomy. Remarkably, this study identified 11 patients who exclusively had autoantibodies. The appearance of autoantibodies without preceding alloantibodies might be attributed to immune system dysfunction resulting from genetic factors, T-reg cell responses to red blood cell autoantigen epitopes mediated by IL-10, and severe hemoglobinopathy (HbE/β thalassemia) [26, 30].

The comparison of hemoglobin levels and transfusion intervals between the two groups in this study did not reveal significant differences. This aligns with the findings of Obeidi *et al.* in an Iranian population, where no relationship was found between pretransfusion hemoglobin levels, transfusion intervals, and the presence of allo-autoantibodies. Similarly, Saifeldeen *et al.*'s study in Egypt reported no significant difference in transfusion intervals between groups with and without allo-autoantibodies [18, 21]. Contrasting results were observed in Essa's research on an Egyptian population, where the group with allo-autoantibodies had shorter transfusion intervals [13]. In addition, Kurniawan *et al.*'s study in Indonesia found that 37.5% of transfusion-dependent beta-thalassemia patients failed to maintain post-transfusion hemoglobin levels for 4 weeks, resulting in shorter transfusion intervals (average 23 days). In this group, the alloantibodies prevalence was 78.6%, and autoantibodies were present in 72.7% of cases [12]. These varied outcomes across studies highlight that the presence of allo- and autoantibodies may not consistently be a singular factor causing a decrease in pre-transfusion hemoglobin levels and a shortening of transfusion intervals in transfusion-dependent beta-thalassemia patients.

Other factors that may influence these outcomes include patient-related factors (such as history of splenomegaly/splenectomy, comorbidities, type of irregular antibodies possessed, psychology, and financial considerations), blood bank factors (such as storage and availability), and transfusion accuracy factors (such as schedule and quantity). Limitations of this research include its single-location focus and limited patient data. Factors other than allo-autoantibodies that can affect hemoglobin levels and transfusion interval still require further research. Researchers suggest the administration of leukoreduced packed red cells (PRC) and antibody screening in pretransfusion testing to mitigate the elevated incidence of allo-autoantibodies.

Conclusion

To sum up, the hemoglobin levels and transfusion intervals in transfusion-dependent beta-thalassemia patients showed no significant differences between the allo- and autoantibody positive and negative groups.

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