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Red blood cell antibody screening and identification in thalassemia major patients

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ABSTRACT

Introduction: Blood transfusion is a medical intervention for thalassemia major to maintain hemoglobin levels between 9-10,5 g/dl. Since it is practically given continuously for life, unexpected complications might occur. Alloimmunization might still happen during transfusion despite being tested for ABO blood group and Rhesus D type and compatibility. Hemolytic reaction as a complication of this transfusion practice would eventually make the patient obtain more frequent blood transfusions. This study aims to examine antibody profiles in thalassemia major patients receiving a long-term regular blood transfusion.

Methods: This was a cross sectional study. Venous blood was drawn in a 10 ml syringe from each patient and then divided into a test tube with anticoagulant and another tube without anticoagulant to prepare plasma and serum. The ABO blood group, Rhesus, and Kell were tested using a tube test, the continued Direct Coombs Test (DCT), and the Rubin test for eluate. Antibody screening and identification using a cell panel were performed for positive results.

Results: 15 (14,29%) of 105 patients were positive for DCT, eluate, screening and antibody identification. This positive result was caused by probable drug-induced (33,36%), warm type AIHA (6,66%), Le^a, Le^b and Jk^b (6,66%).

Conclusions: Alloantibody was rarely found in thalassemia major patients receiving long-term regular blood transfusions.

Keywords: alloimmunization, regular blood transfusion, screening antibody, thalassemia major.
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INTRODUCTION

Thalassemia is a hereditary disorder in the form of a synthesis disorder, globin chain synthesis in either partial or total hemoglobin.[1,2] According to World Health Organization (WHO) in 1994, thalassemia is one of the most common genetic disorders in the world, with approximately 250 million people (4.5% of the world's population) exist.[3] In Indonesia, it is estimated that the frequency of the thalassemia gene is 5%, even in certain regions/ethnicities reaching 10-15%.[4] With a population of approximately 200 million people and a birth rate of 20%, it is estimated that 2,500 babies per year with thalassemia major will be born.[5,6] At the Children's Health Science Thalassemia Center in Dr. Cipto Mangunkusumo Hospital (RSCM) until March 2007, there were 1,264 cases. Registered thalassemia cases throughout Indonesia have only reached 3,000 patients.[5]

The management of thalassemia patients in Indonesia until now has been symptomatic, such as giving blood transfusions.[5] The blood component transfused is Packed Red Cells (PRC), which are given periodically to maintain the patient's hemoglobin concentration in the range of 9-10.5 g/dl, which aims to suppress the erythropoiesis system so that it does not become overloaded. As a result, this forms abnormal Red Blood Cells (RBC).[7–9] The adverse effects of blood transfusion are the occurrence of alloimmunization, reaction to transfusion nonhemolytic debris, Transfusion-related Acute Lung Injury (TRALI), Graft vs. Host Disease (GVHD) and Infectious Diseases Through Cough Blood Transfusion (IDTCBT).[10–13] Alloimmunization reactions occur due to the formation of antibodies to RBC antigens. It is possible when foreign RBC enters the circulation of an individual who does not have the antigen. Apart from blood transfusions, pregnancy can also cause the transfer of RBC antigens between individuals.[10] Exposure to foreign antigens in RBC of thalassemia patients occurs continuously because thalassemia patients, especially those with significant types, will receive blood transfusions for a long time.[14,15] This, over time, causes the patient's immune system to form antibodies against the given RBC antigens. The antibodies included will cause a hemolysis reaction on the RBC that has the relevant antigen. The reaction hemolysis reaction will lead to shorter transfusion intervals of blood, which in turn will strengthen its antigenicity.[16,17]

We can prevent antigen-antibody reactions between the patient's and the donor's blood by screening for antibodies and identifying antibodies in all available blood group systems with their antigens.[18] In Indonesia, screening and identification of antibodies in donor blood and the patient's blood has not been a standardized test. Gantini's research (2005) reported 5% of incompatibility cases in blood transfusion services in Indonesia.[17] Screening and identification of antibodies in RBC aims to determine the presence or absence of antibodies in the plasma/serum of the examined patient.[18] Antibodies that are formed can be natural or immune/acquired. A positive screen

result states that there are antibodies in the blood that are both autoantibodies and alloantibodies. The test then continues with antibody identification. If this is done before blood transfusion, transfusion reactions caused by antigen-antibody reactions will be reduced and may not even be encountered.[19]

In the management of thalassemia primary patients who are periodically receiving blood transfusions, it turns out that there are often results of crossmatch tests, even though there is a match in the ABO and Rh-D blood group test results. It may be due to hemolysis of RBC due to the antigen-antibody reaction because in Indonesia, the mandatory test is only the ABO and Rhesus anti-D systems only. Screening and identifying antibodies in RBC are not routine tests in Indonesia. It is not a common test nor done in thalassemia primary patients who receive repeated transfusions of blood components. In some patients, transfusion reactions occur in heat, chills, and itching after receiving a blood transfusion. This study aimed to determine the antibody profile, distribution, and frequency in patients with thalassemia significant patients who receive repeated blood transfusions so that they can be given compatible blood transfusions.

METHODS

It was a cross-sectional study conducted at the Thalassemia Center, Department of Pediatrics RSCM, in April 2007. Patients with thalassemia major in Thalassemia Center, Department of Pediatrics RSCM were included in this study. Thalassemia patients who required blood transfusion management were taken blood as much as 10 ml with a syringe and first given informed consent. Blood was then separated, some were put into tubes that had been given EDTA anticoagulant, and some were left in the syringe. RBC took the blood in the tube, and then a 5% RBC suspension was made. Furthermore, the blood in the syringe was taken as serum. The data were reported descriptively.

The 5% RBC suspension was subjected to blood grouping (ABO, Rhesus, and Kell blood group systems), Direct Combs Test (DCT), and Eluate (filter test and antibody identification). Serum grouping

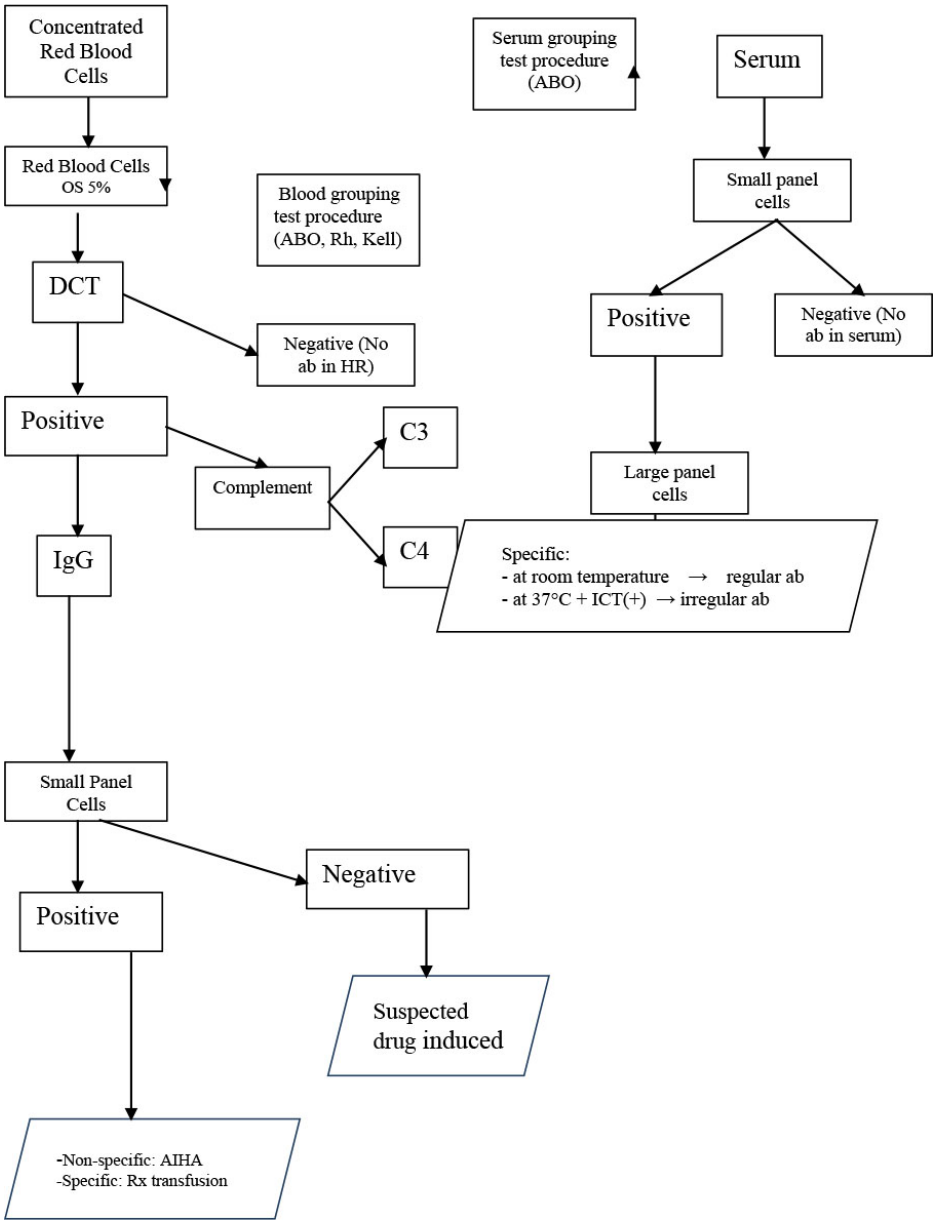

Figure 1. Test flow.

(ABO blood group system), filter test, and antibody identification were performed for the serum. The testing flow of this study is shown in Figure 1.

RESULTS

The results of a study conducted on 105 thalassemia patients who visited the Thalassemia Center of the Pediatrics Department of RSCM during April 2007. The composition of thalassemia patients based on blood type system was O as many as 45 (39%), group A as many as 28 (29%). Group B has as many as 24 (24%), and the last group AB has as many as 8 (8%). It follows the composition of the ABO system blood groups in Indonesia. The frequency of the O gene is the highest Mongoloid genetic marker, especially in the western region of Indonesia, followed by gene B, then gene A, and the least common is gene AB. The least common is the AB gene. All thalassemia patients have the Rh-D(+)/Rh-D(-) blood group system. The composition of patients based on the Kell blood group system was 105 (100%) had the kk phenotype. In other words, no KK and Kk phenotype.

The test with DCT, Eluate, filter test, and antibody identification found that the incompatible patients were 15 (14.29%) and the compatible patients were 90 (85.17%). Conflicting test results (no

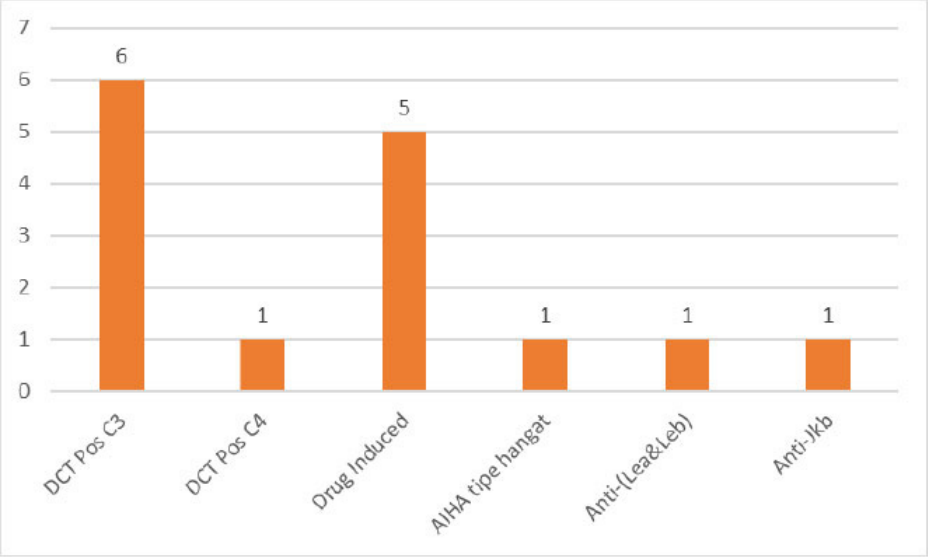

Figure 2. Causes of positive test results in thalassemia patients.

match) with DCT causes Positive C3 as many as 6 (40%), suspected drug-induced as many as 5 (33.36%), and DCT Positive C4, warm type AIHA, anti-(Le^a & Le^b) and anti-JK^b each ((6.66%), as shown in Figure 2.

DISCUSSION

A positive result on the DCT test can detect antibodies or complement present on the surface of RBC. Most Blood Transfusion Units/Blood Banks use one type of antiglobulin that can both IgG and complement and have special reagents that only react with IgG or particular complements, such as C3, C4, or C3d. Slow-type hemolytic transfusion reactions can also cause positive DCT results. It occurs when antigens on the transfused RBC can stimulate the formation of lgG antibodies so that lgG antibodies are attached to the RBC and cause hemolysis.[17] Gantini (2004) also only found 0.0001% Kk phenotype and no KK phenotype in donor blood in Indonesia for Kell's blood group system.[17]

The positive result, which may be caused by drug-induced, is suspected because the follow-up test, in the form of an eluate and filter test and antibody identification, showed negative results. It is because, during incubation, the drugs that coat the RBC will be released. Many drugs can give such results, but in thalassemia patients, it may be caused by antibiotics (Penicillin or Cephalosporins) and analgesics such as mefenamic acid for older patients. Drugs that are thought to coat RBC may activate complement.[15,20]

Warm-type AIHA is caused by IgG class antibodies that usually adhere to the surface of the RBC. IgG can cross the placental barrier, resulting in a cause antigen-antibody reaction during pregnancy, and may lead to HDN. Cold-type AIHA is usually in the form of IgM, which is significant. IgM is generally large and cannot cross the placental barrier.[18]

Positive anti-(Lea & Leb) and anti-Jkb results should concern transfusion management. It should be a concern in transfusion management. Although their antigenicity is less strong than the ABO and Rhesus systems, they can still cause antigen-antibody reactions. Several studies on periodic transfusion in thalassemia primary patients in several countries showed the need to test the Kell blood group system (in addition to the ABO and Rhesus blood group system). Then for Indonesia, it may be necessary to check the Lewis and Kidd blood group systems. Characteristics of the blood group system of thalassemia patients in each country may be unique. Screening tests and identifying RBC antibodies in patients with significant thalassemia patients is vital so that the blood transfusion can provide optimal results for patients who need it.[16] This study has limitations because it is only a descriptive study; thus, it could not evaluate the association of suspected causes.

CONCLUSION

Alloantibodies were sometimes found in thalassemia major patients receiving long term regular blood transfusions. Screening test results and antibody identification in patients with thalassemia primary patients showed that 15 (14.29%) patients were incompatible (no match) between the patient's blood and the donor's blood. Of the 15 subjects, it was found that the cause was DCT Positive C4 as many as 6 (40%), suspected drug-induced 5 (33.36%), positive DCT C3 1 (6.66%), warm type AIHAas much as 1 (6.66%), anti-(Le^a & Le^b) as much as 1 (6.66%), and anti-Jk^b as 1 (6.66%). Further research is needed to evaluate its comprehensive association in higher-level studies.

CONFLICT OF INTEREST

All authors stated that there is no conflict of interest in this study.

ETHICAL CONSIDERATIONS

This study has followed the ICMJE and COPE protocols regarding the research publication ethics guidelines.

AUTHOR CONTRIBUTIONS

All authors stated equal proportions and contributed to this study.

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