p-ISSN 0854-4263
Vol. 22, No. 3, July 2016 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

Editorial Adress:
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Website: http://www.indonesianjournalofclinicalpathology.or.id

Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016

p-ISSN 0854-4263
Vol. 22, No. 3, July 2016 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

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Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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Printed by Airlangga University Press. (OC 202/08.16/AUP-B1E). Kampus C Unair, Mulyorejo Surabaya 60115, Indonesia.
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Thanks to editors in duty of IJCP & ML Vol 22 No. 3 July 2016

Aryati, Ida Parwati, Purwanto AP, July Kumalawati, Puspa Wardhani, Rismawati Yaswir, Kusworini Handono, Ninik Sukartini, Adi Koesoema Aman, Rahayuningsih Dharma, AAG. Sudewa, Sidarti Soehita, Endang Retnowati

INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

2016 July; 22(3): 241–245
p-ISSN 0854-4263 | e-ISSN 4277-4685
Available at www.indonesianjournalofclinicalpathology.or.id

RESEARCH

THE RISK FACTOR OF ALLOANTIBODY FORMATION IN THALASSEMIA PATIENTS RECEIVING MULTIPLE TRANSFUSION

(Faktor Kebahayaan Terbentuknya Aloantibodi pada Pasien Talasemia yang Menerima Transfusi Darah Berulang)

Veronica Fridawati, Teguh Triyono, Usi Sukorini

ABSTRAK

Untuk kelangsungan hidup pasien talasemia intermediet dan mayor, memerlukan transfusi darah secara teratur. Transfusi berulang ini berpeluang membentuk aloantibodi yang dapat menyebabkan kebahayaan hemolitik. Maka transfusi berulang akan memperberat hemolitik karena pada pasien talasemia sudah ada proses tersebut. Tujuan penelitian ini adalah untuk mengetahui berbagai faktor kebahayaan untuk terbentuknya aloantibodi pada pasien talasemia yang mendapat transfusi darah berulang khusus di RSUP Fatmawati, Jakarta. Cara meneliti ini menggunakan rancangan potong lintang. Subjek penelitian adalah semua pasien talasemia yang mendapat transfusi darah berulang di RSUP Fatmawati Jakarta yang memenuhi patokan kesertaan. Sebanyak 81 subjek diikutkan dalam penelitian ini. Data pada penelitian ini di analisis secara statistik dengan uji Chi Kuadrat. Hasil menguji secara Chi Kuadrat menunjukkan: kelamin, suku, diagnosis, selang transfusi darah, jenis darah, reaksi yang terkait, riwayat keluarga, kadar Hb. Kadar feritin dan golongan darah bukan merupakan faktor kebahayaan untuk terbentuknya aloantibodi, sedang faktor usia, jumlah kantong darah yang ditransfusikan, keberadaan komplikasi akibat transfusi darah dan lama masa waktu menerima darah transfusi, merupakan faktor kebahayaan untuk terbentuknya aloantibodi pada pasien talasemia yang mendapat transfusi berulang di RSUP Fatmawati.

Kata kunci: *Talasemia, transfusi darah berulang, faktor kebahayaan untuk terbentuknya aloantibodi*

ABSTRACT

Patients with intermediate and major thalassemia require regular blood transfusions for their survival. Multiple transfusions are potential for the formation of alloantibody, this may increase risk of hemolysis in thalassemia patients. The aim of this study was to know the risk factor of alloantibody formation in thalassemia patients who received multiple transfusions at Fatmawati Hospital, Jakarta. The method of this study used a cross-sectional design. The total subjects included in this study consisted of 81 patients. The data in this study were analyzed statistically with Chi square test. The results of Chi square test showed many factors (gender, ethnicity, diagnosis, interval transfusion, blood type, reaction transfusion, family history, levels of hemoglobin, ferritin levels and blood group) did not belong to the risk factor of alloantibody formation. But the study showed that risk factors such as age, total bags each transfusion, complications due to transfusion and the length duration receiving transfusion, resulted alloantibody formation in thalassemia patients at Fatmawati Hospital.

Key words: Thalassemia, multiple transfusion, risk factor of alloantibody formation

INTRODUCTION

Thalassemia is an abnormality of globin gene marked by the decrease of one or more globin chains. It is derived through autosomal recessive and it is differentiated by its form of homozygote and heterozygote. Homozygote shows severe clinical symptoms and for their survival, patients need regularly blood transfusions. Meanwhile, heterozygote indicates less clinical symptoms and the patient could grow up as an adult.[2] Thalassemia grouping is based on its damaged type of globin chains; there are thalassemia α and β. If the damaged chain is α-type, it is called as thalassemia α; if the damaged chain is β

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type, it is thalassemia β that occurs inside the patient's body. The latter type is commonly found within the population.[2]

Complications that may occur in thalassemia is chronic hemolysis; which could be severe due to post blood transfusion reaction.[4] This complication is caused by the absence or decline of β-type globin chain, therefore it creates unbalanced amount of β-type and α-type globin chains. In this case, unpaired α-type globin will be oxidized and hemichrome. Oxidization makes precipitates attach to erythrocyte membrane; destroying the membrane and causing erythrocyte deformability. This circulates through blood vessels and will be destroyed inside the spleen; therefore, it causes chronic extra vascular hemolytic.

In intermediate and mayor thalassemia patients, an alloantibody is formed as a reaction towards erythrocyte antigen because of blood transfusion. Antibody is valued as a significant clinical factor if it decreases the erythrocyte's lifetime. Therefore, regular blood transfusion cause a worse effect on hemolytic condition.

Alloantibody within the human blood ABO system, called naturally occurring antibodies, is also assigned as the most clinically significant and is formed as IgM. Besides this there are also other immune antibodies caused by blood transfusion (anti-D, anti-K, anti-E, anti-c, anti-Fy, anti-C, anti-Jka, anti-S, anti-Jkb) and usually it is in an IgG form.[4,7] These antibodies and other non-ABOs can not be recognized within cross reaction before blood transfusion. Interaction between antigen and antibody is a secondary immune response that can be detected during 3-7 days after blood transfusion.

Alloimmunization could be reduced by using blood that has less leukocytes component (leucoreduced/ leucodepleted). This mechanism happens because alloimmunization is a presentation of donor erythrocyte's peptide antigen by Antigen Presenting Cells (APC) towards T-Cell receptor (TCR) in CD4 sel T recipients. It is commonly known as T-cell dependent response. It is divided into two ways; direct dan indirect pathway allorecognition. In direct allorecognition, class-II Mayor Hiscompatibility Complex (MHC)/Human Leukocyte antigen (HLA) would be easily and directly recognized by CD4 T-cell recipient. In term, most HLA involved is an unknown HLA. Hence, this reducing leukocytes or leukodepleted process in blood components extensively affect alloimmunization.[9]

The clinical alloantibody towards erythrocytes occurrs more than 30% for those who receive regular blood transfusion; particularly those who receive long-term blood transfusion.[10] Thus, this study aims to the risk factor of alloantibody formation in patients with thalassemia who receive multiple transfusions at Fatmawati Hospital, Jakarta to reduce alloimmunization towards erythrocyte antigens.

METHODS

This research used a cross-sectional design. The population chosen as participants were patients with thalassemia diagnosed by internal medicine specialists as well as pediatricians, taking medical treatment in Fatmawati Hospital and receiving at a minimum 2 times transfusion since they were diagnosed. Exclusion was for patients with thalassemia in the following conditions: being pregnant and organ transplantation program. Total participants fulfilling requirements were 81 patients. This study took place at the Fatmawati Hospital, Jakarta.

The data collection and blood sampling process of 81 patients were done at Blood Transfusion Unit of Fatmawati Hospital. The data comprised patients' personal information such as: age, sex, ethnicity, diagnosis, transfusion interval, blood bags needed in every transfusion, type of blood component needed, other complications as side effect of blood transfusion, their experience of post-transfusion reaction, family medical records and how long they have received transfusion, Hemoglobin and ferritin content, and blood type. The whole blood samples from participants were antibody-filtered simultaneously. Antibody-filtering examination used small panel cell ID DiaCell Diamed (2 filtering panel cells). Big panel cell Diapanel Diamed (10 panel cells) were identified by gel test methods. The principle of Indirect Antiglobulin Test (IAT) was used to examine filtering and antibody identification. The first step was filtering antibody using small panel cell; adding 25 uL of patient's serum in each tube filled with 50 uL panel cell. Then, it was incubated at 37°C during 15 minutes inside the ID incubator. Turned it inside the ID during 10 minutes and read the result. If the result of antibody filtering was positive, it continued to antibody identification. Despite a similarity between identifying and filtering antibody; the former used big panel cells (10 panel cells).[11] To interprete its result, autocontrol was scrutinized to ensure alloantibody or autoantibody formation. If the autocontrol showed a positive result, autoantibody or other substance was formed within the transfused erythrocyte. Further observation was needed to identify the type of autoantibody; IgG or IgM. IgM usually reacted at room temperature.

The following step was to identify the strength of antibody reaction by defining whether it was anti –K, –D, –E, –e, –c and –C. Defining specific features within antibody was figured by ruling out (cross

Table 1. Factors related to alloantibody formation

Characteristics	Alloantibody Negative n=71		Alloantibody Positive n=10		P Value
	N	%	N	%	
Age					0.013
≤6 years	28	34.57	0	0	
>6 years	43	53.08	10	12.35	
Sex					0.108
Female	47	58.02	4	4.94	
Male	24	29.63	6	7.41	
Ethnic					0.430
Betawi	24	29.63	4	4.94	
Sunda	23	28.39	1	1.235	
Java	19	23.45	4	4.94	
Sumatera	1	1.23	0	0	
Aceh	1	1.23	0	0	
Bima	1	1.23	0	0	
Tionghoa (Chinese)	1	1.23	0	0	
Unidentified	1	1.23	1	1.24	
Diagnosis					0.766
Thalassemia α mayor	7	8.64	0	0.00	
Thalassemia β mayor	46	56.79	7	8.65	
Thalassemia β mayor + HbE	18	22.22	3	3.7	
Interval of blood transfusion					1.000
2 weeks	2	2.47	0	0.00	
3 weeks	11	13.6	1	1.24	
4 weeks	58	71.6	9	11.11	
Blood bags needed					0.003
1 bags	24	29.63	0	0.00	
2 bags	37	45.68	4	4.94	
3 bags	1	1.23	1	1.24	
4 bags	9	11.1	5	6.17	
Type of blood component					0.292
Packed Red Cell (PRC)	63	77.78	1	1.23	
Washed Red Cell (WRC)	8	9.88	9	11.11	
Complications caused by transfusion					0.001
No	71	87.65	6	7.41	
Yes	0	0	4	4.94	
Post reaction transfusion					0.443
No	51	62.96	6	7.41	
Yes	20	24.69	4	4.94	
Family medical record					0.587
No	63	77.77	10	12.35	
Yes	8	9.87	0	0.00	
Years receiving blood transfusion					0.008
Minimum - <1 year	5	6.17	0	0.00	
1 years - <5 years	29	53.8	0	0.00	
5 years - <10 years	16	19.75	2	2.47	
10 years – maximum	21	25.92	8	9.88	
Hemoglobin level					1.000
≤7.6	39	48.15	5	6.175	
>7.6	32	39.50	5	6.175	
Ferritin level					1.000
≤300	2	2.47	0	0.00	
>300	69	85.18	10	12.35	
Blood type					0.962
Blood type: O	24	29.63	4	4.94	
Blood type: A	20	24.69	2	2.47	
Blood type: B	21	25.92	3	3.70	
Blood type: AB	6	7.41	1	1.24	

one that shows positive antigen in panel cell, do not cross heterozygote antigen) and circling antigen that was not crossed in the same row, considering common temperature in reactive antibody. Thus, at the minimum, there will be three reacted as positive erythrocyte antigen and three negative which not react at all. The last step, data would be analyzed statistically through Chi-Square test and reported in table form.

RESULTS AND DISCUSSION

This study analyzed several variables as follows: age, sex, ethnic, dignoses, blood transfusion interval, blood pouch needed in every transfusion, type of blood component transfused, other diseases as the effect of transfusion, reaction experience after blood transfusion, family medical record and how long has been received transfusion, the amount of hemoglobin and ferritin and blood type.

To analyze antibody formation, it was related to variables aforementioned, summed based on positive alloantibody in 10 subjects of this research. Here are the results of statistically analysis by Chi-Square test (Table 1).

Table 1 showed by Chi-Square test, those factors: sex, ethnic, diagnoses, blood transfusion interval, blood bags needed in every transfusion, type of blood component transfused, other diseases as the effect of transfusion, reaction experience after blood transfusion, family medical record and how long has received transfusion, the amount of hemoglobin and ferritin; are not significant factors towards alloantibody formation. It is supported by the p value >0.05, this result is similar to studies conducted by Sadeghian *et al* and Obeidi *et al*.[13–14] However, type of blood component; all patients received Washed Red Cell (WRC) with positive or negative alloantibody conveys different result. WRC transfusion in Fatmawati Hospital is usually given if patients suffer additional reaction after blood transfusion such as itchy skin, fever and dizziness. From the table, the interpretation of p=0.292 means there is no significant difference between patients receiving WRC with positive alloantibody and those with negative alloantibody. Simply put, with or without WRC, alloantibody formation still occurs. It could be explained by erythrocyte antigen agglutinates in erythrocyte in WRC production; thus it forms alloantibody. These factors: age, blood bags needed and other diseases caused by blood transfusion and how long the patients receive transfusion are significant factors towards alloantibody formation.

There is a remarkable difference between the patients below 6 years and older, in terms of the formation of alloantibody. Those who are older than 6 years old are more exposed to erythrocyte antigen caused by recurring blood transfusion; this is in according with a previous study by Blaney and Howard.[15] There are factors affecting immunogenity of erythrocyte; one of it is the dose and density of antigen. It informs how much erythrocytes enter the body as well as its antigen in which increases the possibility of immune's response.[15]

The following variable that informs a significant result is blood bags needed in every transfusion. In this study, alloantibody formation occurred in patients with thalassemia who have received between 2–4 blood bags for every transfusion. It is aligned to the study by Singer *et al*.[16] Similar result applies to the persons experiencing disease after blood transfusion compared to those who do not suffer. In this study, there were patients experiencing side effects through splenectomy. Sadeghian *et al*[13] also found that patients with splenectomy have a higher alloantibody. It happened because the absence of spleen increases risk as the aftermath of alloantibody formation. Besides, a significant difference occurred in patients who received blood transfusions during or more than 5 years. A research by Sadeghian *et al*[13], Sirchia; cited by Bilwani *et al*[17], argued that patients suffering major thalassemia would produce alloantibody within their blood cells after 6 years receiving regular blood transfusion. Some even argue that alloantibody would be produced only after 10 years receiving regular blood transfusion if patients have sensibility in an earlier stage.[13,17]

CONCLUSION AND SUGGESTIONS

Some dangerous factors triggering alloantibody formation have been acquired in patients with thalassemia receiving regular transfusion in Fatmawati Hospital. This condition applies to: 6-year-old patients or older who received 2–4 blood bags in every transfusion; or those who suffer diseases after transfusion; or those who have received transfusion during or more than 5 years.

For further research, more participants and blood sampling are needed to obtain better validities towards the study. Moreover, it would be useful to prevent alloantibody effectively.

If there is any possibility to decrease the risk of alloantibody formation, it could be done through: patients who should receive blood from a permanent donor, they should be transfused with leucodepletion. The blood transfusion should be done before the hemoglobin level is very low in order to prevent greater amounts of blood in each transfusion.

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