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Erythrocyte Antibody Due to Alloimmunization in Repeated Transfusion: A Meta-Analysis

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Abstract

BACKGROUND: Blood transfusion is one form of life-saving efforts to improve health. Each individual will experience a different transfusion reaction. Having a history of repeated transfusions increases the risk of alloimmunization leading to the development of erythrocyte alloantibodies.

AIM: This study is a meta-analysis of various studies on erythrocyte antibodies due to alloimmunization in repeated transfusion.

METHODS: Literatures were searched through the PubMed, DOAJ, and Google Scholar databases using the keywords "repeated transfusion," "alloimmunization," and "erythrocyte antibody" published in 2017 - 2021. All identified articles were then screened for relevance as well as duplication according to inclusion and exclusion criteria. Then, the articles were analyzed using software review manager 5.4 and software comprehensive meta-analysis (CMA) version 3.

RESULTS: A total of seven articles were included in this study. Based on the analysis, we found that there was no association between alloimmunization in repeated transfusions with erythrocyte antibodies based on gender (pooled odds ratio 1.00 [95% CI 0.70 - 1.42].

CONCLUSION: Alloimmunization on repeated transfusion was not significantly associated with erythrocyte antibody based on gender.

Introduction

Blood transfusion is one form of life-saving efforts to improve health. A person requires a blood transfusion because of blood loss or lack of important components in the blood. There are various kinds of blood components, such as PRC (packed red cell), RBC (red blood cell), TC (thrombocyte concentrate), FFP (fresh frozen plasma), washed erythrocyte, cryoprecipitate, and platelets. Some patients require blood transfusions, especially erythrocyte components during the course of the disease or even throughout their lives [1].

Blood transfusion is a medical procedure that is generally very safe to do, since before being transfused the blood is tested, handled, and stored carefully. However, it is still possible for a recipient to have a transfusion reaction. Transfusion reactions can be divided into two types: Acute transfusion reactions that occur within the first 24 h after transfusion and delayed transfusion reactions that occur within more than 24 h or 1.5 months after transfusion. Delayed transfusion reactions include delayed hemolytic reactions, post-transfusion purpura, iron overload and transmissible infections caused by transfusion. Transfusion reactions can also be divided into three categories based on complaints and signs, including: mild reactions such as local pruritus, localized rash, and urticaria; moderate reactions such as palpitations, mild shortness of breath, and headache; severe reactions such as chest pain, back pain or groin pain, headache, hypotension, and tachycardia [2], [3].

Each individual will experience a different transfusion reaction. This is caused by differences in susceptibility, immunological response, age, gender, diagnosis, types of blood components, and blood incompatibility. It is also influenced by a history of repeated transfusions. A study by Eko Putri Rahajeng in 2020 explained that there were 72.1% (72 cases) of subjects who had a history of the previous transfusions experiencing transfusion reactions. Individuals who have a history of repeated transfusions will experience sensitization which causes the formation of alloantibodies in the recipient's body due to exposure to HLA (human leukocyte antigen) or HPA (human platelet antigen) through previous transfusions or commonly referred to as alloimmunization. The interaction of donor antigens with previously sensitized recipient antibodies triggers systemic inflammation with the release of the cytokine interleukin-1 and tumor necrosis factor (IL-1, TNF) [2].

Alloimmunization is the result of exposure to an antigen through a previous blood transfusion, tissue transplant, or pregnancy. In addition, it is also influenced by the lack of phenotypic compatibility between donor blood and recipient blood [4]. Alloimmunization is the result of exposure to an antigen through a previous blood transfusion, tissue transplant, or pregnancy. In addition, it is also influenced by the lack of phenotypic compatibility between donor blood and recipient blood [4]. The frequency of alloimmunization in patients with repeated transfusions varies in different studies. In thalassemia patients, the alloimmunization frequency is 4–50%, in hemato-oncological patients 1.9–13% patients are alloimmunized, and in kidney disease patients the alloimmunization frequency is 1.27–13.1% [5].

The risk of alloimmunization increases with exposure to erythrocytes. As many as 30% of patients who received repeated transfusions had erythrocyte alloantibodies. Another study found that 60% of individuals with a history of chronic transfusion were alloimmunized. Half of these individuals have antibodies to more than one antigen, so obtaining suitable blood is very difficult and can even lead to delayed hemolytic transfusion reaction (DHTR) and sometimes life threatening. Therefore, it is highly recommended to perform phenotype matching between donor and recipient erythrocytes to avoid sensitization in chronic transfusion-dependent patients [4].

Meta-analysis is a statistical technique that combines and analyzes two or more research results quantitatively [6]. A meta-analysis aims to obtain an estimate of the effect size or the strength of the relationship as well as the magnitude of the difference between variables, test hypotheses (p value) or estimates (confidence interval), and also control variables that have the potential as confounding factors so as not to interfere with the statistical significance of a study [7], [8]. One of the advantages of meta-analysis is the formation of new studies that have a large number of subjects so that the conclusions obtained are more definitive. Even so, meta-analysis has a weakness in technical problems, which is the choice of appropriate statistics for combining data [8].

Based on the description above, we intended to conduct a meta-analysis of erythrocyte antibodies due to alloimmunization in repeated transfusion, which aims to obtain a more definitive research conclusion.

Methods

This is a quantitative research with a meta-analysis study design. The source of this research data was obtained through literature searches on the internet through the PubMed, DOAJ, and Google Scholar databases. The literature search is limited to only research conducted in the 2017–2021 range. In this study, communication with related researchers or manual tracing was not carried out. The keywords used in the search were a combination of keywords related to “repeated transfusion,” “alloimmunization,” and “erythrocyte antibody.”

The studies included in this meta-analysis were pre-selected based on clear inclusion and exclusion criteria. The inclusion criteria for this study included: A study that examined the association of alloimmunization in repeated transfusion with erythrocyte antibodies, a cohort or case–control study design, full text, written in English, in the period of 2017–2021, and had sufficient data to be analyzed. Meanwhile, the exclusion criteria for this study were: studies with alloimmunization death outcomes on repeated transfusions, anonymous studies, not in full text, not having enough data to analyze, and duplicated studies or previously published studies.

Information obtained from each study can be in the form of raw data, exposure, and outcome. The data are converted into a uniform tabular format, such as: Year of publication, location, design, exposure, definition of exposure, and outcome of each study. Data analysis used a fixed-effect model or a random-effect model. In the fixed-effect model, it is assumed that the variability among various studies is only based on the opportunity factor, meaning that if the research is carried out unlimited, the same results will be obtained so that the confidence interval obtained is narrow. Meanwhile in the random-effect model, things that are taken into account are the intra-study variability and inter-study variability. With this technique, a wider confidence interval will be obtained compared to the fixed-effect model [8]. The software used to perform the meta-analysis is Review Manager 5.4. The results of data were presented in a forest plot graph to describe the combined effect size of each of the variables studied. Meanwhile, publication bias was tested using funnel plot, Egger’s test and trim and fill technique in comprehensive meta-analysis (CMA) software version 3.

Sensitivity tests were conducted to prove whether the results of the meta-analysis were relatively stable with respect to changes. The sensitivity test was carried out in this study by comparing the results when analyzed using the fixed-effect model with the results analyzed by the random-effect model. If the results are the same or nearly the same, it can be concluded that the variation between studies is not significant in the data set.

Results

Search results in the PubMed, DOAJ, and Google Scholar databases identified 22,341 articles. Articles were reviewed on the title and 22,233 articles

were excluded from the study. The selection results based on abstracts excluded as many as 90 articles. There were only 18 articles can then be reviewed based on the full text. A total of 11 articles were excluded based on full text. Finally, there were seven articles remained to be meta-analyzed. The results of the study selection can be seen on the flow chart (Figure 1).

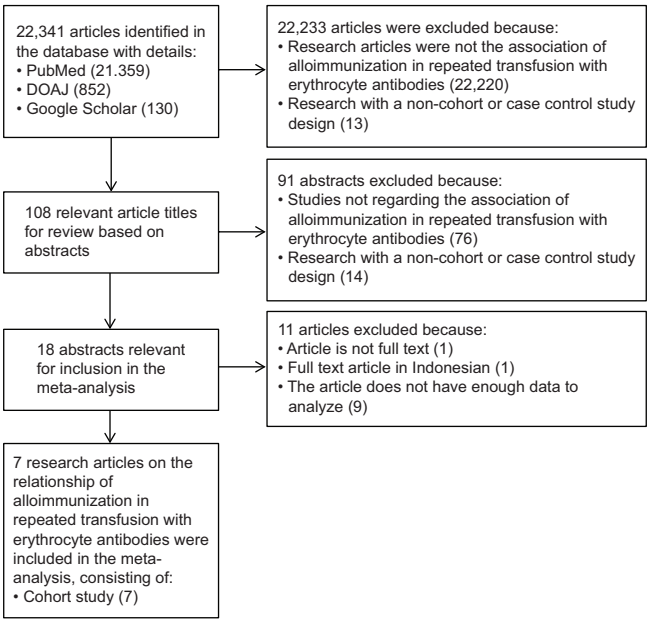

Figure 1: Results of article selection of erythrocyte antibodies due to alloimmunization in repeated transfusion

A total of seven research articles were included in the meta-analysis with cohort study design. The studies were extracted into a table containing information about each article (Table 1).

Meta-analysis of erythrocyte antibody due to alloimmunization in repeated transfusion

There were seven research articles on erythrocyte antibodies due to alloimmunization in repeated transfusion included in the meta-analysis and analyzed using the random effect model analysis model (Figure 2). The results of the analysis showed that the research variation was heterogeneous, with $p < 0.05$ in the heterogeneity test ($p = 0.006$) and the value of variation between studies (I^2) of 55%. In the forest plot of the association of alloimmunization to repeated transfusions with erythrocyte antibodies, $p > 0.05$ was obtained ($p = 1.00$) with a pooled odds ratio of 1.00 (95% CI 0.70 - 1.42). It can be concluded that there was no association between alloimmunization in repeated transfusions with erythrocyte antibodies based on gender.

Publication bias of erythrocyte antibody due to alloimmunization in repeated transfusion

Publication bias in this study was tested using a funnel plot and Egger's test. Trim and fill technique was also used to see the possibility of missing articles that cause the funnel plot to be asymmetrical. The funnel plot showed a symmetric distribution of the studies, so it can be concluded that publication bias does not affect the association of erythrocyte antibodies due to alloimmunization in repeated transfusions. In the Egger's regression test, the intercept value was equal to zero (-0.00515) which indicated the effect of publication bias on the association of erythrocyte antibodies due to alloimmunization on repeated transfusions was very small. In the trim and fill test using the missing studied to the left side of the mean effect and missing studied to the right side of the mean effect parameter, it was found that there was no difference between pooled OR before and after trim and fill. It can be concluded that publication bias does not affect the association of erythrocyte antibodies due to alloimmunization in repeated transfusions. In addition, there was no change in the value of the pooled ratio between the

Table 1 : Critical appraisal of research articles meta-analysis of erythrocyte antibodies due to alloimmunization in repeated transfusions

No	Researcher (year of study)	Journal/database	Study design	Research location	Research subject	Total sample	Percentage /m/OR/RR
1	Das SS, *et al*. (2021)	Global Journal of Transfusion Medicine/DOAJ	Cohort	India	Patients with hemoglobinopathy, hemato-oncology diseases, chronic kidney disease with repeated transfusion	4350 (1506 males; 2844 females)	p=0.0008
2	Handa A, *et al*. (2020)	Journal of Family Medicine and Primary Care/DOAJ	Cohort	India	Patients with history of repeated transfusion and negative initial antibody screening	100 (36 males; 64 females)	p=0.040
3	Neto OGDV, *et al*. (2018)	Hematology, Transfusion and Cell Therapy/DOAJ	Cohort	Brazil	Patients with hemoglobinopathy, hemato-oncology diseases, chronic kidney disease with repeated transfusion	153 (74 males; 79 females)	p=0.6915
4	Jariwala K, *et al*. (2019)	Indian Journal of Medical Research/Google scholar	Cohort	India	Patients with sickle cell disease dan major β-thalassemia	471 (271 males; 200 females)	p<0.001
5	Abdulqader AMR, *et al*. (2020)	Korean Journal of Clinical Laboratory Science/Google scholar	Cohort	Iraq	Patients with major β-thalassemia	204 (106 males; 98 females)	p=0.293
6	Bhuva DK, *et al*. (2017)	Asian Journal of Transfusion Science/DOAJ	Cohort	India	Patients with major β-thalassemia, sickle cell disease, chronic kidney disease, postpartum hemorrhage, aplastic anemia, myelodysplastic syndrome with more than 10 transfusions	300 (178 males; 122 females)	p=0.9122
7	Waheed U, *et al*. (2019)	Global Journal of Transfusion Medicine/DOAJ	Cohort	Pakistan	Patients with major β-thalassemia having more than 10 transfusions	475 (259 males; 216 females)	p=0.7999

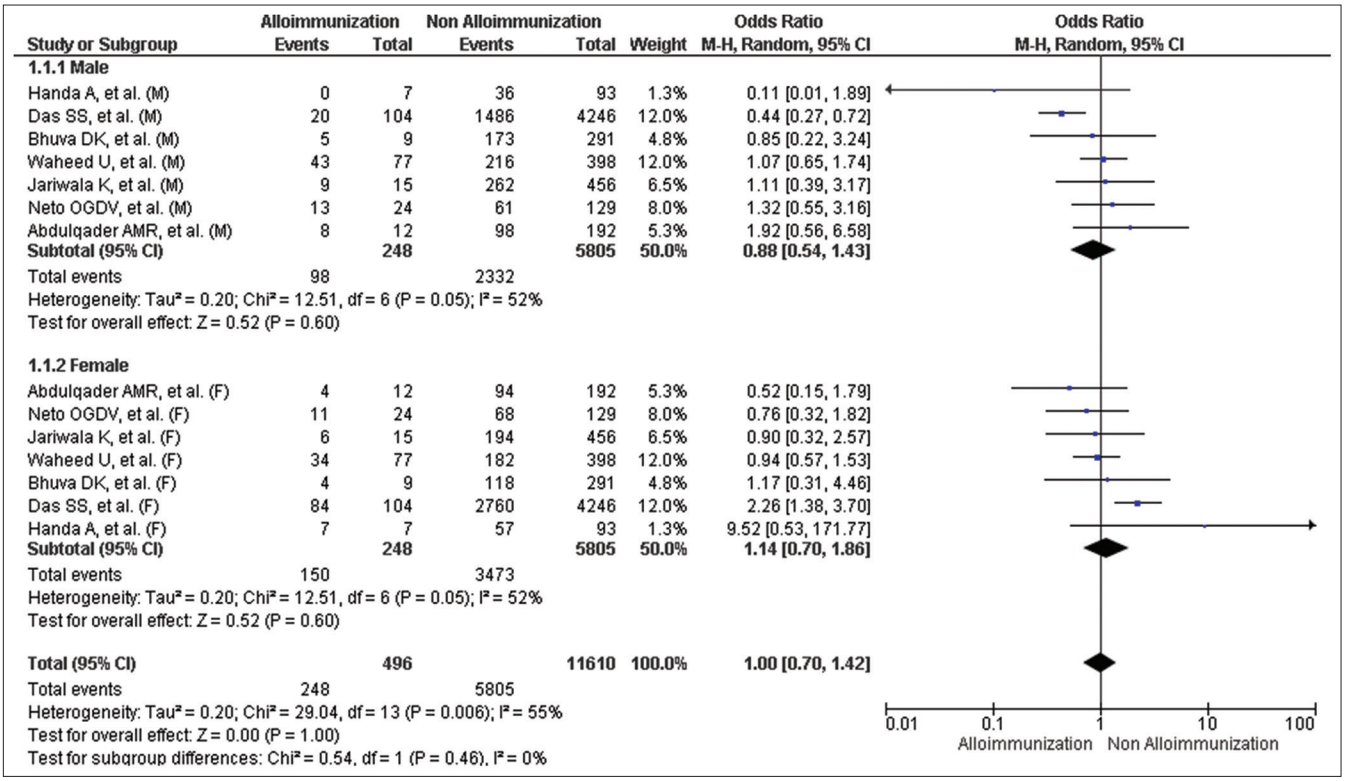

Figure 2: Forest plot of erythrocyte antibodies due to alloimmunization in repeated transfusion

observed values and the adjusted values, which were both 1.00 (0.70 - 1.42).

Sensitivity test

The sensitivity test was done by comparing the fixed-effect model with the random-effect model. Sensitivity testing was very limited due to the very small number of studies. The results of the sensitivity test showed that on the alloimmunization variable, the inter-variation had a significant effect. There was a decrease in the pooled OR value from the fixed-effect model [1.00 (0.81 - 1.23)] to the random-effect model [1.00 (0.70 - 1.42)] and the wider of the confident interval.

Discussion

A total of seven research articles were included in the meta-analysis. All research articles are articles with a cohort study design. All articles contain the association of alloimmunization with erythrocyte antibodies in repeated transfusion based on gender in diseases requiring more than one transfusion. All articles only include p values, so the OR/RR values were obtained from the data available in the research articles.

The risk estimates and study characteristics were extracted from the original study. The statistical method used to determine the pooled OR ratio of alloimmunization in repeated transfusion with erythrocyte antibodies was seen from the heterogeneity test results. The heterogeneity test of the alloimmunization relationship on repeated transfusion with erythrocyte antibodies gave the result p = 0.006. It can be concluded that the variation between studies is heterogeneous so that the statistical method used is the random effect model. The results of the meta-analysis of the association of alloimmunization in repeated transfusions with erythrocyte antibodies showed that there was no association of alloimmunization in repeated transfusions with erythrocyte antibodies by sex with a pooled OR ratio of 1.00 (95% CI 0.70 - 1.42). We concluded that erythrocyte antibodies are not associated with alloimmunization in repeated transfusions in either male or female.

The results in this study are in accordance with research conducted by Abdulqader *et al*. in 2020 on 204 major β-thalassemia patients. In this study, it was found that there was no significant relationship between gender and the incidence of alloimmunization in patients with major β-thalassemia with p value of 0.293 [9]. The results of this study are also supported by the research by Bhuva *et al*. in 2017 which stated that there were 5 of 178 male patients and 4 of 122 female patients who experienced alloantibodies after repeated transfusions. This result was not significant after statistical analysis with p value obtained is p = 0.9122 [10].

Distinct from this study, research conducted by Das *et al*. in 2021 stated that erythrocyte antibodies due to alloimmunization in repeated transfusions

were significantly higher in women than men with the incidence of 84 cases out of 2844 women. They also stated that gender is one of the factors that affect alloimmunization in patients with repeated transfusions with an odds ratio of 2.2613 (95% CI 1.38 - 3.69) and p = 0.0011 [5]. Another study by Handa *et al*. in 2020 described that women who had repeated transfusions were more susceptible to alloimmunization than men with a p value of 0.040. This is because women who suffer from anemia and having pregnancy are important risk factors for alloimmunization [11].

Erythrocyte alloimmunization is an immune response to foreign red blood cell antigens that generally occurs after sensitization due to blood transfusion and pregnancy. The risk factors for the occurrence of red blood cell alloantibodies depend on the age, gender, and genetic factors of the patient as well as the number and frequency of transfusions that have been performed. Patients who receive repeated transfusions due to various causes such as hemoglobinopathies, hematological diseases, various types of cancer, organ transplantation, and renal failure have an alloimmunization prevalence of up to 60% [11].

Alloimmunization can lead to difficulty in matching blood, decreased erythrocyte survival, and increased transfusion requirements. In addition, it can cause multiple organ failure, electrolyte disturbances, coagulopathy, and in some cases death. Therefore, detection or screening of erythrocyte antibodies is important in the treatment of transfusion. This screening can be done routinely at specified time intervals after the transfusion is performed. This screening is expected to help reduce the incidence of erythrocyte alloimmunization and delayed hemolytic transfusion reactions in multi-transfusion patients [11].

The publication bias test was carried out using a funnel plot and Egger's test. This test was conducted to examine the potential bias in the association of alloimmunization in repeated transfusion with erythrocyte antibodies due to relevant studies but not included in the meta-analysis. The funnel plot as well as the results from Egger's test concluded that there was no publication bias in the association of alloimmunization in repeated transfusion with erythrocyte antibodies.

Trim and fill tests were performed to confirm the presence of publication bias in the association of alloimmunization in repeated transfusion with erythrocyte antibodies. By assuming that there were studies with insignificant results or studies that were on the left and right of the center line on the funnel plot, we conclude that zero research articles are missing. There was no publication bias in the association of alloimmunization in repeated transfusion with erythrocyte antibodies. This was possible because the missing relevant research was research with the results of the association of alloimmunization in repeated transfusions with erythrocyte antibodies. So that publication bias with the assumption that articles with insignificant research results did not affect the meta-analysis of the association of alloimmunization in repeated transfusion with erythrocyte antibodies.

The sensitivity test carried out in this study was to compare the fixed-effect model with the random-effect model. In the association of alloimmunization in repeated transfusions with erythrocyte antibodies, heterogeneity test was obtained with P = 0.006 so that the analytical method used was a random effect model where it was assumed that the alloimmunization relationship with repeated transfusions with erythrocyte antibodies was susceptible to changes. If we assumed that the variation between studies is homogeneous, then the pooled OR ratio was 1.00 (95% CI 0.81 - 1.23). Alloimmunization variables were analyzed using the random effect model method because the research was heterogeneous where the variability between studies on the variables was more diverse or more numerous and makes the confidence interval range wider.

Research limitation

There was no communication with the researcher, resulting in several articles that could not be analyzed because the data displayed were inadequate. Articles that have to be paid to get the full text were also a drawback in this study, due to limited costs. The research articles that were incorporated in the meta-analysis were the combination of research articles with a cohort design. This was a drawback in itself considering the strength of the relationship that is evidenced by the different designs.

Conclusion

Alloimmunization on repeated transfusion was not associated with the occurrence of erythrocyte antibodies based on gender with an estimated effect of 1.00. There was no influence of publication bias on the association of alloimmunization in repeated transfusion with erythrocyte antibodies as evidenced by a symmetric funnel plot, the intercept value of Egger's test was equal to zero and no missing articles were found based on the trim and fill technique.

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