Prevalence and specificity of red blood cell alloantibodies and autoantibodies in transfused Iranian β-thalassemia patients: A systematic review and meta-analysis

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Abstract:
BACKGROUND: Repeated allogeneic blood transfusions in thalassemia major patients stimulate the patient’s immune system to generate antibodies against foreign erythrocyte antigens. This study was carried out to systematically review the findings of available studies about the prevalence of alloantibodies and autoantibodies, as well as the type of causative antigens among transfusion-dependent thalassemia patients in Iran.

METHODS: Electronic search was conducted on Medline, PubMed, Cochrane, EMBASE, ScienceDirect, and Persians databases. All relevant articles published from January 1990 to July 2018 were included. Abstracts of conference booklets which that been published in the last 5 years were also included in the meta-analysis. The search language was restricted to English and Persian. The quality of studies was evaluated according to a checklist developed by authors, and Cochrane Risk of Bias Assessment Tool was used to evaluate the risk of bias.

RESULTS: Twenty-three relevant articles met all the inclusion criteria. The prevalence of alloimmunization was 13%. Our study showed that anti-D (25%) and anti-K (25%) were most prevalent among Iranian β-thalassemia patients. Data analysis shows the autoantibody prevalence to be 1% among 3787 patients. Meta-regression revealed that the prevalence of alloantibodies increases with each year as the average age of the study population increases.

CONCLUSION: The prevalence of red blood cell (RBC) alloantibodies in transfused Iranian β-thalassemia patients was high. Appropriate preventive strategies such as RBC phenotyping for patients before beginning transfusion and using extended RBC donor–recipient matching, specifically for Rh and Kell system, could be implemented to avoid complications in thalassemia patients.

Keywords:
Alloantibodies, alloimmunization, autoantibodies, red blood cell, splenectomy, thalassemia

Introduction
Thalassemia is the most common inherited form of hemolytic anemia worldwide. It is caused by reduced synthesis of one or two globulin chains due to partial or complete deficiency of either α- or β-globin chain synthesis and subsequently results in abnormal formation of hemoglobin. Thalassemias are classified to α- or β-thalassemia based on which chain of the globulin is involved. Thalassemia is commonly reported in Mediterranean basin and Middle East (Turkey, Iran), as well as Arabian Peninsula and Southeast Asia.
These patients need early and regular blood transfusions.\textsuperscript{9,10} Frequent blood transfusion decreases the complications of severe anemia and extends survival in patients.\textsuperscript{11} Chronic blood transfusion as the main supportive treatment is crucial for patients with homozygous beta-thalassemia to sustain growth and development during childhood and to maintain acceptable quality of life during adulthood.\textsuperscript{12,13} Repeated allogeneic blood transfusions in thalassemia major patients stimulate the patient’s immune system to produce antibodies against foreign erythrocyte antigens.\textsuperscript{14-16} One of the main complications of chronic transfusions is red blood cell (RBC) alloimmunization.\textsuperscript{17} The most important adverse effect of the alloimmunization is the increased need for transfusion due to hemolysis and restriction in obtaining further compatible blood for transfusion, both of which can significantly complicate transfusion management for transfusion-dependent patients.\textsuperscript{18,19}

Risk factors known to affect the incidence of alloimmunization include female sex, history of pregnancy, duration of transfusion therapy, antigen immunogenicity, genetic and environmental factors, and racial differences between recipients and donors.\textsuperscript{20,21} In addition, patients with a history of antibodies after a few blood transfusions present a greater risk for additional alloantibodies and hemolytic transfusion reactions.\textsuperscript{22} The main alloantibodies reported in thalassemic patients are directed against Rhesus (Rh), Kell, Kidd, and Duffy systems.\textsuperscript{22,23}

Alloimmunization to these clinically significant antigens may lead to difficulty in finding compatible blood units.\textsuperscript{24} Clinical significance of alloantibodies depends on titer, specificity, immunoglobulin class, and clinical context.\textsuperscript{25}

Developing antibodies against RBCs is an inevitable consequence of repeated transfusion. Although preventive matching for the highly immunogenic Rh-K antigens is applied for transfusion-dependent patients and successfully reduces the high immunization incidence, antibodies may develop as a result of other unmatched blood group transfusions.\textsuperscript{18,26,27} Therefore, early detection of alloantibodies is extremely important as blood transfusion is crucial for thalassemia patients.\textsuperscript{28}

Considering the fact that Iran is located on the belt of thalassemia, the prevalence of thalassemia is relatively high in Iran.\textsuperscript{29} Since limited information is available about the incidence of immunization in such patients, this study was carried out to systematically review the findings of available studies about the prevalence of alloantibodies and autoantibodies, as well as the type of causative antigens among transfusion-dependent thalassemia patients in Iran. The other objective of this study was to explore potential sources of heterogeneity in the findings of different studies.

Methods

Literature search strategy

Electronic search was conducted on Medline, PubMed, Cochrane, EMBASE, ScienceDirect, Sid, Iran Medex, and Magiran databases with the following MeSH search headings: Alloantibodies, Isoimmunization, Isoantibodies, Alloantigens, Antigen, Erythrocyte, and Red blood cell. In addition to MeSH search headings, other words were applied in the search strategy as well, which included Alloimmunization, Alloimmune, Antibodies, RBC, and the names of provinces of Iran. All relevant articles published from January 1990 to July 2018 were included in the present study. The articles were also identified using hand searching of the references of the studies. Abstracts of conference booklets that have been published in the last 5 years were also included in the meta-analysis. The search language was restricted to English and Persian. One researcher (H. Rostamian) applied the selection criteria to the titles and abstracts of all articles identified by the search strategy; any studies irrelevant to our objectives were excluded in the first step.

Study selection

Articles were eligible only if they reported the frequency of RBC alloantibodies in thalassemic patients in Iran. Two independent authors reviewed each full text of potentially relevant articles. They individually decided whether the article should be included or excluded on the basis of predefined inclusion/exclusion criteria checklist conducted by the authors. Disagreements between the two authors were settled by discussion and reaching consensus. In case disagreement persisted, third author was consulted to resolve the disagreement and took the final decision.

Quality evaluation

The checklist was precisely developed through consensus among all authors to evaluate methodological quality of studies. The quality of studies was evaluated based on dichotomous question and according to following parameters: study objectives, sample collection method, population size determination, characteristics of the study population, detailed inclusion/exclusion criteria, data collection method, along with the validity, explicit findings, and appropriate data analysis methods of the studies, as well as analysis of confounding factors. Checklist allowed grading of the articles on a scale of 1–10. Nonqualified studies (articles with grades below 3) were excluded from the study. Besides, duplicated citations were not included. We also assessed the risk of bias in the included studies by Cochrane Risk of Bias Assessment Tool.\textsuperscript{30} This included random sequence generation, allocation concealment, selective outcome reporting, completeness of outcome data, blinding of
participants, personnel, outcome assessors, completeness of outcome data, and other potential sources of bias. These domains were assessed independently by two reviewers. Any differences of opinion have been settled through consensus or through consultation with a third party.

**Data extraction**

After determining the eligible papers, data were extracted from selected studies according to a standard protocol, and subsequently, all relevant data were tabulated. To improve accuracy and critical appraisal, data extraction was conducted by two independent researchers, and disagreements between researchers were resolved by discussion and reaching consensus. The extracted data are listed in Table 1.

**Statistical analysis**

We used metaprop command using STATA version 14.1 (StataCorp, College Station, TX, USA) for pooling proportions. We fit the logistic-normal random-effects model and the exact method to the data. With these model and method, there is no worry about studies with cure rates close to or at 1 in some studies and multiple studies with widely variable sample sizes being used. To fit the generalized linear mixed model, the updated command metaprop_one was used.\(^{31,32}\)

We calculated 95% confidence intervals (CIs) by using score statistic and the exact binomial method and Freeman–Tukey double arcsine transformation of proportions via ftt option. Heterogeneity of the prevalence estimates between studies was decided by Q statistic and Tau\(^2\) index. Forest plots were drawn displaying the variation of the alloimmunization prevalence and each alloantibody among all studies together with the pooled measure and subgroup analysis.

The association between alloantibody and autoantibody proportion and age, male/female proportion, quality score, splenectomy proportion, and location of subject of studies (whether they are located in coastal cities or not) was assessed with univariate and multiple meta-regression analysis.

**Results**

At the beginning of the web search, we first identified 374 articles based on titles and abstracts related to our research topic. Out of which, 130 were duplicates and 69 were not about thalassemia which was removed. 12 articles were removed because their language was no English or Persian. In addition, we also excluded reviews, letters, comments, or case reports.

Finally, full texts of 97 articles were assessed. 62 were excluded because these articles did not contain data about Iranian beta-thalassemia patients and these patients were not the main focus of their studies. In the end, only 23 relevant articles meeting all the inclusion criteria were selected for data extraction and quantitative analysis. Figure 1 shows our search and selection process and also the reasons for exclusion.

Of the 23 studies, 5734 individuals were included in our study. Studies were conducted in all regions of Iran, though many were in Tehran [Table 2]. Pooling of these studies yielded overall proportions of 13 (95% CI: 0.10, 0.17) per 100 transfused individuals. The highest and lowest prevalence of alloimmunization was reported in Sari (40%) and Zahedan (0%), respectively,\(^{38,40}\) which is shown in Figure 2.

**Red blood cell alloantibody specificities**

Regarding the results from studies, the majority of alloantibodies belonged to the Rh and Kell
| Author         | Year | City of study    | Sample size | Mean age | Number of male | Number of female | Number of alloimmunization | Number of autoantibodies | Number of splenectomy | Overall bias | Reference |
|---------------|------|------------------|-------------|----------|----------------|------------------|-----------------------------|----------------------------|------------------------|-------------|-----------|
| Sadeghian et al. | 2009 | Mashhad          | 313         | 22.6     | 187.00         | 126.00           | 9.00                        | ND                         | 101.00                 | Low         | [33]      |
| Karimi et al.  | 2007 | Shiraz           | 711         | ND       | 366.00         | 345.00           | 38.00                       | 12.00                      | 203.00                 | Low         | [34]      |
| Mirzaeian et al.| 2013 | Zahedan          | 385         | 22.5     | 221.00         | 164.00           | 69.00                       | 21.00                      | 28.00                  | Low         | [35]      |
| Kiani et al.   | 2006 | Khoram abad      | 65          | 17.63    | 35.00          | 30.00            | 1.00                        | 0.00                       | 17.00                  | Unclear     | [36]      |
| Hiradfar et al.| 2015 | Ahvaz            | 133         | 13       | 66.00          | 67.00            | 25.00                       | 17.00                      | 28.00                  | High        | [37]      |
| Eshghi         | 2003 | Zahedan          | 163         | 14.42    | 96.00          | 67.00            | 0.00                        | 0.00                       | 21.00                  | Unclear     | [38]      |
| Tahannejad-Asadi et al. | 2013 | Ahvaz            | 70          | 22.5     | 31.00          | 39.00            | 6.00                        | ND                         | 18.00                  | Low         | [39]      |
| Kosaryan et al.| 2014 | Sari             | 218         | 19.6     | 100.00         | 118.00           | 88.00                       | ND                         | ND                     | Unclear     | [40]      |
| Ansari et al.  | 2009 | Tehran           | 458         | 17.7     | 221.00         | 237.00           | 49.00                       | 1.00                       | ND                     | High        | [41]      |
| Azarkeivan et al. | 2015 | Tehran           | 441         | 14.4     | 234.00         | 207.00           | 50.00                       | 1.00                       | 222.00                 | Low         | [42]      |
| Shamsian et al.| 2008 | Tehran           | 121         | 19.56    | 55.00          | 66.00            | 9.00                        | ND                         | 10.00                  | Unclear     | [43]      |
| Ansari and Moshtaghiyan | 2008 | Tehran           | 80          | 13.8     | 37.00          | 43.00            | 3.00                        | 15.00                      | 80.00                  | Unclear     | [44]      |
| Rahgozar et al.| 2005 | Esfahan          | 52          | 18.75    | 36.00          | 16.00            | 40.00                       | ND                         | 9.00                   | Unclear     | [45]      |
| Azarkeivan et al. | 2011 | Tehran and Gazvin| 835         | 8.35     | 416.00         | 419.00           | 101.00                      | 1.00                       | 346.00                 | Low         | [46]      |
| Ahmadi         | 2000 | Kermanshah       | 142         | 13       | ND             | ND               | 13.00                       | ND                         | ND                     | High        | [47]      |
| Obedi et al.   | 2011 | Bushehr          | 90          | 16.96    | 39.00          | 51.00            | 9.00                        | ND                         | ND                     | Unclear     | [48]      |
| Vaziri et al.  | 2015 | Yazd             | 100         | 16.5     | 46.00          | 54.00            | 4.00                        | ND                         | ND                     | Low         | [49]      |
| Farsinejad     | 2002 | Kerman           | 300         | 14.97    | ND             | ND               | 27.00                       | ND                         | ND                     | High        | [50]      |
| Mahbod         | 2003 | Tehran           | 300         | ND       | 180.00         | 120.00           | 31.00                       | 2.00                       | ND                     | High        | [51]      |
| Ghezelbash     | 2015 | Ardebil          | 106         | 44       | ND             | ND               | 9.00                        | 3.00                       | ND                     | High        | [52]      |
| Moghaddas Ghafarokhy | 2013 | Chaharmahal Bakhtiar | 234       | 16.1     | 130.00        | 104.00           | 12.00                       | ND                         | ND                     | High        | [53]      |
| Ghasemi et al. | 2016 | Tehran           | 110         | ND       | 62.00          | 48.00            | 12.00                       | 2.00                       | ND                     | High        | [54]      |
| Esmaeili       | 2012 | Tehran           | 307         | 9.8      | 164.00         | 143.00           | 132.00                      | ND                         | ND                     | High        | [55]      |

ND=No available data
blood group systems [Table 3]. Our study showed that anti-D and anti-K were the most prevalent alloantibodies among Iranian β-thalassemia patients, and the prevalence of each of them has been reported at 25%, followed by anti-C, E, Lua, and Coa (13%), as shown in Table 3.

Some articles reported two alloantibodies in one individual, among which the highest prevalence was D + C (9%, 95% CI: 5–12, I² = 97%) [Table 4].

**Alloantibody prevalence by sex**

In 12 studies, the prevalence of antibodies was reported by sex [Table 5]. The prevalence of alloimmunization according to forest plot in Figure 3 was 11% among females, and according to forest plot in Figure 4, the prevalence was 10% among males.

**Prevalence of autoantibody**

Twelve studies also reported autoantibodies’ prevalence. Data analysis shows the autoantibody prevalence to be 1% among 3787 patients (95% CI: 0–3, Tau² = 2.12).

**Prevalence of splenectomy**

1092 cases out of the 3469 patients underwent splenectomy. In this meta-analysis, the incidence of splenectomy in 13 studies was 9% (95% CI: 5–16, Tau² = 0.73).

**Meta-regression analysis**

The results of univariate, and multiple weighted, linear meta-regression analysis are presented in Tables 6 and 7. Unadjusted meta-regression revealed that the prevalence of alloantibodies increases by 1.2% (95% CI: 0.3–2.3; P = 0.02) with each year as the average age of the study population increases. This higher rate remained marginally significant after adjustment of male/female ratio, quality score, splenectomy proportion, and location of subjects of studies. In addition, unadjusted meta-regression revealed that the prevalence of alloantibodies increased by 2.6% (95% CI: 3–4.8; P = 0.03) with every increased unit of male/female ratio of the study population. This higher rate remained significant after adjustment of age, quality score, splenectomy proportion, and location of subjects of studies.

As shown in Table 7, there was no association between autoantibody proportion and age, male/female proportion, quality score, splenectomy proportion, and location of subjects of studies.

**Discussion**

The results of this comprehensive meta-analysis reveal that 13% of transfused thalassemia patients in Iran bear clinically significant RBC alloantibodies, and anti-D and anti-K were found to be the most prevalent antibodies.
Our findings revealing high occurrence of alloantibodies against antigens of Rh and Kell systems, both of which are highly immunogenic antigens, are in line with the results of previous studies. [56-58] Antibodies are most commonly
Table 5: Prevalence of red blood cell alloimmunization in β thalassemia patients by sex

| Sex     | Number of studies | Sample size | Tau² | Overall estimate (%) | 95% CI |
|---------|-------------------|-------------|------|----------------------|--------|
| Male    | 11                | 1522        | 1.57 | 5                    | 2-12   |
| Female  | 11                | 1389        | 0.83 | 8                    | 5-15   |

CI=Confidence interval

Figure 4: Forest plot of red blood cell alloimmunization prevalence in males

Table 4: Prevalence of multiple red blood cell alloantibodies

| Alloantibody | Number of studies | Sample size | Number of alloantibodies | Tau² | Overall estimate (%) | 95% CI |
|--------------|-------------------|-------------|--------------------------|------|----------------------|--------|
| D + C        | 6                 | 251         | 22                       | 0.001| 9                    | 6-13   |
| D + E        | 3                 | 157         | 3                        | 0.001| 2                    | 1-6    |
| K + D        | 2                 | 151         | 2                        | 0.001| 1                    | 0-5    |
| K + E        | 2                 | 151         | 2                        | 0.001| 1                    | 0-5    |
| K + Kpa      | 3                 | 200         | 5                        | 0.2  | 3                    | 1-7    |

CI=Confidence interval

Another systematic review article that contains 41 studies from different regions of the world indicated that the prevalence of RBC alloimmunization was 11.4% and the alloantibodies were mostly against Rh (52.4%) and Kell (25.6%) systems. Because this study includes many articles from Iran and the Middle East, the reported prevalence in this study is close to ours.

As a risk factor sex remains a contentious issue. Due to being more commonly exposed to immunizing events, i.e. pregnancy and transfusion, women are expected to bear a higher rate of RBC alloimmunization. However, the present study did not identify any noticeable differences in prevalence of RBC alloimmunization between thalassemic males and females, which was found to be 10% and 11%, respectively. Also, in line with our findings, a number of studies have achieved the same results. Surprisingly, unadjusted meta-regression showed a 2.6% increase of alloantibodies prevalence with every unit increase of

in transfused patients, indicating the necessity to more comprehensively study weak D antigens and D variants.

Developed against K antigen in Kell blood system, while anti-D, -E, and -C antibodies are most frequently found in Rh blood system.

In a meta-analysis study executed in sub-Saharan Africa, the RBC alloimmunization prevalence in transfusion recipients was 6.7% (95% CI: 5.7–7.8), which is almost half the prevalence found on our population. Several factors, such as genetic background, sex, and racial differences among studies, can affect RBC alloimmunization, which perhaps explains the differences between our studies and those of the studies in Africa. Despite the present policies of D antigen-matched transfusion in both Iran and sub-Saharan Africa, meta-analyses conducted in both these regions reveal a high anti-D antibody occurrence.
The prevalence of RBC alloantibodies in transfused Iranian β-thalassemia patients was high, but the prevalence of autoantibodies was not higher than other countries. Clinically significant alloantibodies and autoantibodies can impact life quality and overall survival of patients with beta-thalassemia major. Appropriate preventive strategies such as RBC phenotyping for patients before beginning transfusion and using extended RBC donor–recipient matching, specifically for Rh and Kell antigens system, could be implemented to avoid complications in these patients. Our findings implicate further research, clinical practice, and policymaking in the field and that clinicians should remain aware of the importance of prevention as a priority.

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**Conflicts of interest**
There are no conflicts of interest.

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