Review Article

Access this article online
Quick Response Code:

Website: www.ajts.org
DOI: 10.4103/ajts.AJTS_39_20

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

Hosein Rostamian, Ehsan Javandoost[1], Mozhdeh Mohammadian[1,2], Abbas Alipour[3]

Department of Medical Immunology, School of Medicine, Tehran University of Medical Sciences, [1]Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, [2]Department of Medical Laboratory, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, [3]Department of Community Medicine, Thalassemia Research Center, Medical Faculty, Mazandaran University of Medical Sciences, Sari, Iran

Address for correspondence:
Dr. Abbas Alipour, Mazandaran University of Medical Sciences, Sari, Imam Sq., Joybar 3way, Start of Valiye Asr Highway, P. O. Box: 44157-33971, Sari, Iran.
E-mail: alipour.abbas59@gmail.com

Submitted: 31-03-2020
Revised: 18-10-2020
Accepted: 29-03-2021
Published: 26-05-2022

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.[1] 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.[2] Thalassemias are classified to α- or β-thalassemia based on which chain of the globulin is involved.[3,4] Thalassemia is commonly reported in Mediterranean basin and Middle East (Turkey, Iran), as well as Arabian Peninsula and Southeast Asia.[5-8]

How to cite this article: Rostamian H, Javandoost E, Mohammadian M, Alipour A. Prevalence and specificity of red blood cell alloantibodies and autoantibodies in transfused Iranian β-thalassemia patients: A systematic review and meta-analysis. Asian J Transfus Sci 2022;16:111-20.

These patients need early and regular blood transfusions.[9,10] Frequent blood transfusion decreases the complications of severe anemia and extends survival in patients.[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.[12,13] Repeated allogeneic blood transfusions in thalassemia major patients stimulate the patient's immune system to produce antibodies against foreign erythrocyte antigens.[14-16] One of the main complications of chronic transfusions is red blood cell (RBC) alloimmunization.[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.[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.[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.[22] The main alloantibodies reported in thalassemic patients are directed against Rhesus (Rh), Kell, Kidd, and Duffy systems.[22,23]

Alloimmunization to these clinically significant antigens may lead to difficulty in finding compatible blood units.[24] Clinical significance of alloantibodies depends on titer, specificity, immunoglobulin class, and clinical context.[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.[18,26,27] Therefore, early detection of alloantibodies is extremely important as blood transfusion is crucial for thalassemia patients.[28]

Considering the fact that Iran is located on the belt of thalassemia, the prevalence of thalassemia is relatively high in Iran.[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 predecided 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.[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.

Table 1: All relevant data extracted from studies

Data items	Details of data
General information	First author's name; study location; study date; definition used for alloimmunization
Population characteristics	Sex groups; mean age; age range
Methodological information	Sampling method; sample size; scope of study
Studyoutcomes	Prevalence of alloimmunization; type of alloantibodies; prevalence of each alloantibody; number of patients with alloantibody; number of patients with history splenectomy; average of blood transfusion duration in patients; average of blood transfusion intervals in patients; method of alloantibody type assessment

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

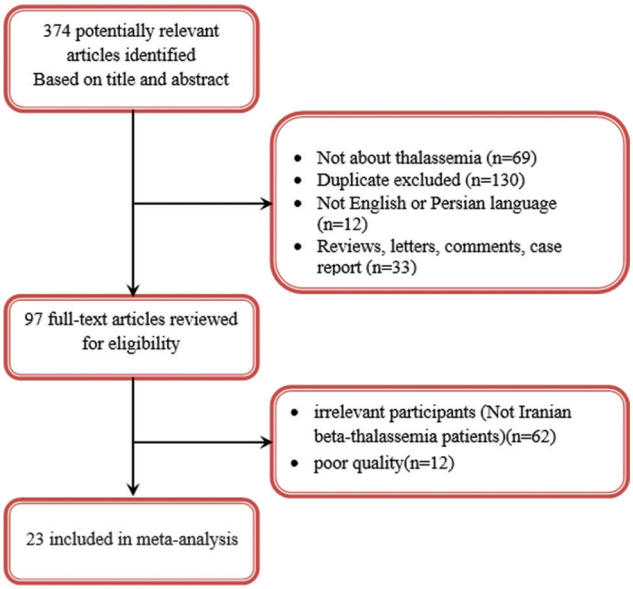

Figure 1: Study selection and exclusion process

Table 2: Articles on prevalence of red blood cell alloimmunization in Iran

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 Moshtaghian	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 Qazvin	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]
Obeidi *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	9.00	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	Cheharmahal Bakhtiari	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

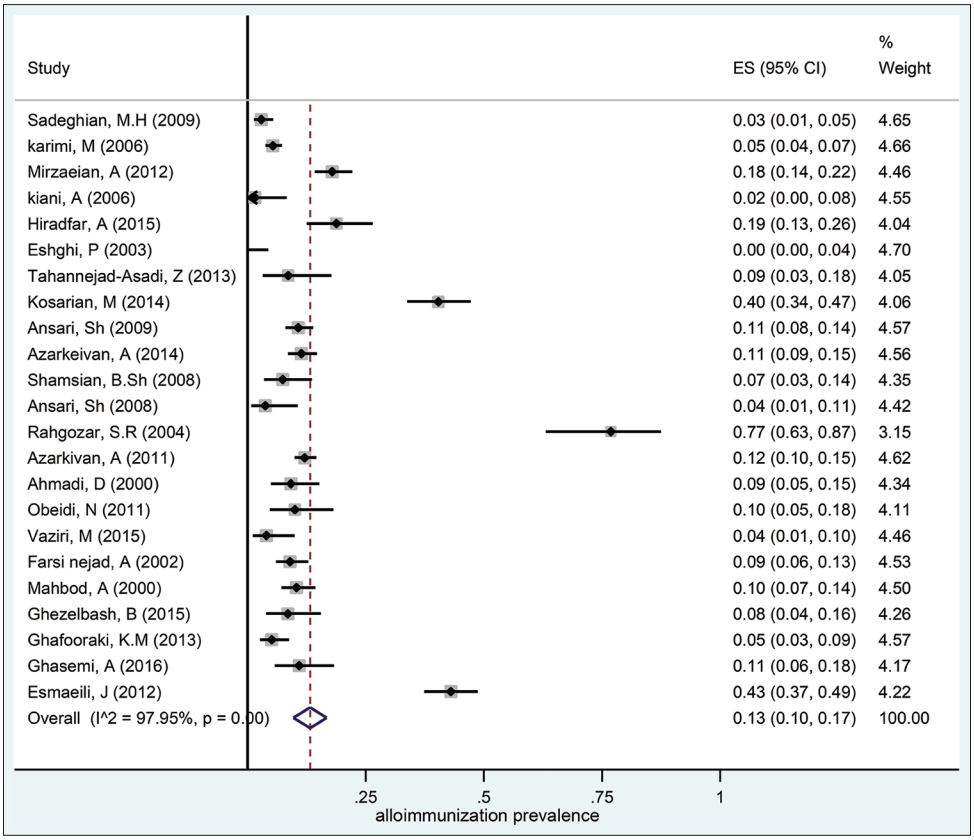

Figure 2: Forest plot of red blood cell alloimmunization prevalence in Iranian thalassemia patients with 95% confidence interval

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, I^2 = 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, Tau^2 = 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, Tau^2 = 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.

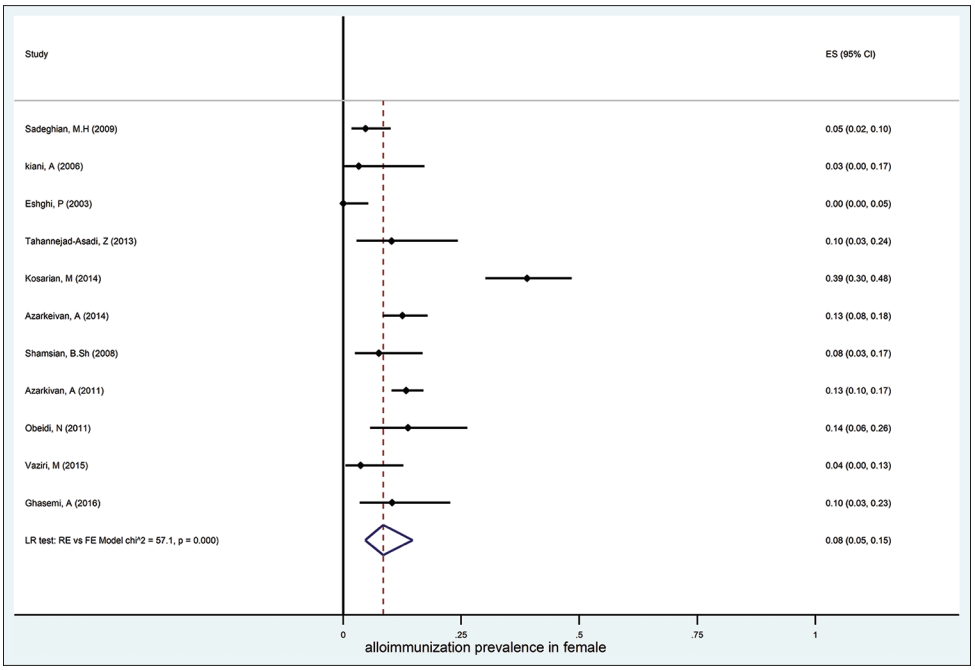

Figure 3: Forest plot of red blood cell alloimmunization prevalence in females

Table 3: Red blood cell alloantibodies prevalence in Iranian thalassemia patients

Blood group	RBC alloantibodies	Number of studies	Sample size	Number of alloantibodies	Tau²	Overall estimate (%)	95% CI
Rh	D	13	409	82	0.39	24	16-33
	C	13	503	77	0.5	13	8-20
	E	17	521	79	0.07	15	11-19
	c	9	419	28	0.001	7	5-10
	e	6	386	12	0.03	3	2-6
	CW	7	409	54	0.97	10	5-22
Kell	K	13	355	74	0.36	24	17-34
	k	2	157	2	NA	1	0-5
	Kpa	3	219	10	0.001	5	2-8
	Kpb	1	88	1	NA	1	-
	Jsa	1	9	1	NA	11	-
	Jsb	1	88	1	NA	1	-
Duffy	Fya	3	109	7	0.001	6	3-13
	Fyb	4	206	10	0.001	5	3-9
Lewis	Lea	4	121	39	0.46	21	8-43
	Leb	2	157	24	0.001	15	10-22
Lutheran	Lua	5	216	32	0.39	12	6-23
	Lub	1	88	1	NA	1	-
MNSS	M	3	159	14	0.001	9	5-14
	N	3	229	16	0.83	6	2-17
	S	3	137	12	0.02	9	4-18
	s	3	197	10	0.001	5	3-9
Kidd	jka	4	301	19	0.24	6	3-11
	jkb	2	157	12	0.001	8	4-13
Xg	Xja	2	157	12	0.001	8	4-13
Colton	Coa	1	88	1	NA	13	0.7-2
	Cob	2	151	4	NA	2	0-5

RBC=Red blood cell, CI=Confidence interval, Rh=Rhesus, NA=Not available

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

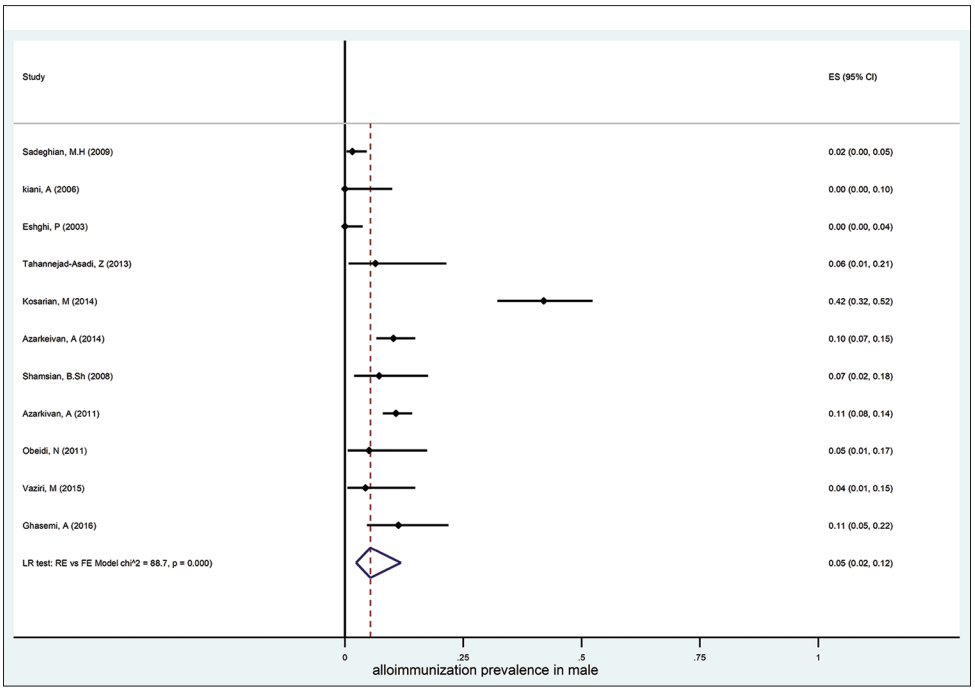

Figure 4: Forest plot of red blood cell alloimmunization prevalence in males

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

Table 5: Prevalence of red blood cell alloimmunization in 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

developed against K antigen in Kell blood system, while anti-D, -E, and -C antibodies are most frequently found in Rh blood system.[59,60]

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.[61] 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 in transfused patients, indicating the necessity to more comprehensively study weak D antigens and D variants.[61,62]

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.[63] 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.[64] 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.[65,66] Surprisingly, unadjusted meta-regression showed a 2.6% increase of alloantibodies prevalence with every unit increase of

Table 6: Meta-regression results for univariate and multiple (adjusted effect) models assessing the effect of age, male: female ratio, quality score, splenectomy proportion, and study location on alloantibody proportion

	Unadjusted		Adjusted	
	β (95% CI)	*P*	β (95% CI)	*P*
Age	0.012 (0.003-0.023)	0.02	0.025 (−0.002-0.05)	0.07
Male:female ratio	0.26 (0.03-0.48)	0.03	0.3 (0.05-0.56)	0.03
Quality score	−0.01 (−0.04-0.02)	0.47	−0.04 (−0.11-0.03)	0.19
Splenectomy proportion	−0.14 (−0.65-0.37)	0.57	0.097 (−0.31-0.51)	0.6
Study location	0.03 (−0.14-0.2)	0.75	−0.012 (−0.22-0.19)	0.89

CI=Confidence interval

Table 7: Meta-regression results for univariate and multiple (adjusted effect) models assessing the effect of age, male: female ratio, quality score, splenectomy proportion, and study location on autoantibody proportion

	Unadjusted		Adjusted	
	β (95% CI)	*P*	β (95% CI)	*P*
Age	−0.004 (−0.011-0.04)	0.34	−0.003 (−0.02-0.02)	0.56
Male:female ratio	−0.09 (−0.27-0.086)	0.28	−0.25 (−0.82-0.32)	0.2
Quality score	−0.002 (−0.02-0.012)	0.76	−0.002 (−0.05-0.05)	0.9
Splenectomy proportion	0.12 (−0.099-0.33)	0.23	0.13 (−0.29-0.54)	0.32
Study location	0.03 (−0.04-0.1)	0.32	0.11 (−0.08-0.3)	0.13

CI=Confidence interval

male/female ratio. Such result suggests the significance of considering other factors such as a history of pregnancy in females, age at the beginning of transfusion, number of transfused blood units, and status of splenectomy, all of which are varying in different studies.

Patients with thalassemia intermedia and major need frequent transfusions to make up for deficiency of well-normal functioning red cells.[67] Owing to the fact that defective or damaged RBCs are eliminated in the spleen, thalassemia patients develop an enlarged hyperfunctioning spleen. Removing the spleen, therefore, might prolong RBCs survival, which in turn could reduce the need for transfusions.[68] The incidence of splenectomy among Iranian thalassemia patients was found to be 10% in our study. Patients with splenectomy have a reduced risk of alloimmunization in comparison with patients without it.[69] Our meta-regression analysis found no correlation between splenectomy and RBC alloimmunization. Another systematic review investigating the safety and efficacy of splenectomy in patients of beta-thalassemia was unable to find any hard evidence.[68] We report 1% autoantibody prevalence among 3787 patients, which is close to prevalence reported in other countries.[59] No association was found between autoantibody incidence and age, quality score, splenectomy proportion, male/female proportion, and location of subjects of studies with meta-regression analysis. The number of transfused blood units increased with older age; thus, it could be considered as an RBC alloimmunization risk factor. Meta-regression revealed a 1.2% increase of alloantibody incidence with each added year as the average age of the study population, which is consistent with some other studies.[70]

Conclusion

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.

Financial support and sponsorship

This study is financially supported by the Research Deputy of Mazandaran University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

References

1. Kim Y, Park J, Kim M. Diagnostic approaches for inherited hemolytic anemia in the genetic era. Blood Res 2017;52:84-94.
2. Forget BG, Bunn HF. Classification of the disorders of hemoglobin. Cold Spring Harb Perspect Med 2013;3:a011684.
3. Roberts DJ. Transfusion medicine – The way ahead. Transfus Med 2011;21:215-6.
4. Koohi F, Kazemi T, Miri-Moghaddam E. Cardiac complications and iron overload in beta thalassemia major patients – A systematic

review and meta-analysis. Ann Hematol 2019;98:1323-31.
5. Chao YH, Wu KH, Lu JJ, Shih MC, Peng CT, Chang CW. Red blood cell alloimmunisation among Chinese patients with β-thalassaemia major in Taiwan. Blood Transfus 2013;11:71.
6. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, *et al*. β-Thalassemia distribution in the old world: An ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis 2017;9:e2017018.
7. Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS, *et al*. Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfus Med 2006;16:200-3.
8. Voskaridou E, Kattamis A, Fragodimitri C, Kourakli A, Chalkia P, Diamantidis M, *et al*. National registry of hemoglobinopathies in Greece: Updated demographics, current trends in affected births, and causes of mortality. Ann Hematol 2019;98:55-66.
9. Prati D. Benefits and complications of regular blood transfusion in patients with beta-thalassaemia major. Vox Sang 2000;79:129-37.
10. Zafari M, Kosaryan M, Gill P, Alipour A, Shiran M, Jalalli H, *et al*. Non-invasive prenatal diagnosis of β-thalassemia by detection of the cell-free fetal DNA in maternal circulation: A systematic review and meta-analysis. Ann Hematol 2016;95:1341-50.
11. Vichinsky E, Neumayr L, Trimble S, Giardina PJ, Cohen AR, Coates T, *et al*. Transfusion complications in thalassemia patients: A report from the Centers for Disease Control and Prevention (CME). Transfusion 2014;54:972-81.
12. Chonat S, Quinn CT. Current standards of care and long term outcomes for thalassemia and sickle cell disease. Adv Exp Med Biol 2017;1013:59-87.
13. Rebulla P. Blood transfusion in beta thalassaemia major. Transfus Med 1995;5:247-58.
14. Pandey H, Das SS, Chaudhary R. Red cell alloimmunization in transfused patients: A silent epidemic revisited. Asian J Transfus Sci 2014;8:75-7.
15. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood 2000;96:3369-73.
16. Stiegler G, Sperr W, Lorber C, Fabrizii V, Höcker P, Panzer S. Red cell antibodies in frequently transfused patients with myelodysplastic syndrome. Ann Hematol 2001;80:330-3.
17. Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122:1062-71.
18. Khan J, Delaney M. Transfusion support of minority patients: Extended antigen donor typing and recruitment of minority blood donors. Transfus Med Hemother 2018;45:271-6.
19. Ogedegbe HO. A review of immune mediated transfusion reactions. Lab Med 2002;33:287-95.
20. Gehrie EA, Tormey CA. The Influence of clinical and biological factors on transfusion-associated non-ABO antigen alloimmunization: Responders, hyper-responders, and non-responders. Transfus Med Hemother 2014;41:420-9.
21. da Cunha Gomes EG, Machado LA, de Oliveira LC, Neto JFN. The erythrocyte alloimmunisation in patients with sickle cell anaemia: A systematic review. Transfus Med 2019;29:149-61.
22. Strobel E. Hemolytic transfusion reactions. Transfus Med Hemother 2008;35:346-53.
23. Fasano RM, Chou ST. Red blood cell antigen genotyping for sickle cell disease, thalassemia, and other transfusion complications. Transfus Med Rev 2016;30:197-201.
24. Sood R, Makroo RN, Riana V, Rosamma NL. Detection of alloimmunization to ensure safer transfusion practice. Asian J Transfus Sci 2013;7:135-9.
25. Körmöczi GF, Mayr WR. Responder individuality in red blood cell alloimmunization. Transfus Med Hemother 2014;41:446-51.
26. Pirenne F, Yazdanbakhsh K. How I safely transfuse patients with sickle-cell disease and manage delayed hemolytic transfusion reactions. Blood 2018;131:2773-81.
27. Baía F, Correia F, Alves B, Martinez F, Koch C, Carneiro A, *et al*. Phenotyping Rh/Kell and risk of alloimmunization in haematological patients. Transfus Med 2016;26:34-8.
28. Bayanzay K, Alzoebie L. Reducing the iron burden and improving survival in transfusion-dependent thalassemia patients: Current perspectives. J Blood Med 2016;7:159-69.
29. Abolghasemi H, Amid A, Zeinali S, Radfar MH, Eshghi P, Rahiminejad MS, *et al*. Thalassemia in Iran: Epidemiology, prevention, and management. J Pediatr Hematol Oncol 2007;29:233-8.
30. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, *et al*. Updated guidance for trusted systematic reviews: A new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst Rev 2019;10:ED000142.
31. Schwarzer G, Chemaitelly H, Abu-Raddad LJ, Rücker G. Seriously misleading results using inverse of Freeman – Tukey double arcsine transformation in meta-analysis of single proportions. Res Synth Methods 2019;10:476-83.
32. Nyaga VN, Arbyn M, Aerts M. Metaprop: A Stata command to perform meta-analysis of binomial data. Arch Public Health 2014;72:39.
33. Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, *et al*. Alloimmunization among transfusion-dependent thalassemia patients. Asian J Transfus Sci 2009;3:95-8.
34. Karimi M, Nikrooz P, Kashef S, Jamalian N, Davatolhagh Z. RBC alloimmunization in blood transfusion-dependent beta-thalassemia patients in southern Iran. Int J Lab Hematol 2007;29:321-6.
35. Mirzaeian A, Tamaddon G, Naderi M, Hosseinpour M, Sargolzaie N. Prevalence of alloimmunization against RBC antigens in thalassemia major patients. Zahedan J Res Med Sci 2013;15:2013;15(7):e92916.
36. Kiani A, Abdi J, Shirkhani Y, Kashi M. Prevalence of alloimmunization against RBC antigens in thalassemia major patients of Lorestan province in 1383. The Scientific Journal of Iranian Blood Transfusion Organization 2006;3:265-71.
37. Hiradfar A, Keikhai K, Pedram M. Clinical prevalence and dominant patterns of alloimmunization in transfusion-dependent thalassemia patients at Ahvaz Shafa hospital. Persian J Med Sci 2015;1(1):18-22.
38. Eshghi P. Evaluation of alloimmunization in major B. thalassemic patients in Zahedan in 2001. J Mazandaran Univ Med Sci 2003;13:36-42.
39. Tahannejad-Asadi Z, Elahi A, Mohseni A, Talebi M, Khosravi M, Jalalifar MA. Screening and identifying of erythrocyte alloantibodies in patients with Thalassemia major referred to Ahvaz Shafa hospital. KAUMS Journal (FEYZ). 2013 May 10;17(2):165-72.
40. Kosaryan M, Mahdavi M, Hojjati M, Roshan P. Prevalence of alloimmunization in major beta thalassemia in northern Iran (2010). J Gorgan Univ Med Sci: 2014;16(3):76-9.
41. Ansari S, Azarkivan A, Salahmand M, Lotfi P. Assessment of alloimmunization in multi transfuse (Thalassemia) patients admitted in Ali Asghar Children's Hospital during 2004-05. Razi J Med Sci 2009;16:65-72.
42. Azarkeivan A, Ahmadi MH, Zolfaghari S, Shaiegan M, Ferdowsi S, Rezaei N, *et al*. RBC alloimmunization and double alloantibodies in thalassemic patients. Hematology 2015;20:223-7.
43. Shamsian BS, Arzanian MT, Shamshiri AR, Alavi S, Khojasteh O. Frequency of red cell alloimmunization in patients with β-major thalassemia in an Iranian referral hospital. Iran J Pediatr 2008;18:149-53.
44. Ansari S, Moshtaghian PV. Assessment of frequency of alloimmunization and erythrocyte autoimmunization in

transfusion dependent thalassemia patients. Acta Med Iran 2008;46(2):137-40.
45. Rahgozar S, Moafi A, Yavari F, Hourfar H. Alloantibody detection in major Beta Thalassemic patients transfused within less-than-20-day intervals. Scientific Journal of Iran Blood Transfus Organ. 2005;1(2):1-9.
46. Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, *et al*. Blood transfusion and alloimmunization in patients with thalassemia: Multicenter study. Pediatr Hematol Oncol 2011;28:479-85.
47. Ahmadi D. Prevalence of alloantibodies in patients with beta-thalassemia major (Kermanshah, 2000). J Kermanshah Univ Med Sci 2001;1:50-5.
48. Obeidi N, Mankhian AR, Hatami G, Emami H. Antibody screening in patients with thalassemia major. Lab Med 2011;42:618-21.
49. Vaziri M, JavadzadehShahshahani H, Moghaddam M, Taghvaee N. Prevalence and specificities of red cell alloantibodies in transfusion-dependent beta thalassemia patients in Yazd. Iran J Ped Hematol Oncol 2015;5:93-9.
50. Farsinejad AR. Evaluation of immune antibody against RBCs of Thalassemic Patients in Kerman Province. Modares J Med Sci (Pathobiology) 2002;5:93-9.
51. Mahbod AS. Evaluation of alloantibodies against red blood cells in transfusion dependent thalassemia patients. In: Congress of the Iranian Society for Oncology and Hematology and the Annual Cancer Nursing Conference. Tehran: Laboratory & Diagnosis 2003.
52. Ghezelbash B. Evaluation of Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients in Ardabil hospitals. In: International Congress on Blood; Diseases and Clinical Administrations. Yasuj: Yasuj University of Medical Sciences; 2015.
53. Moghaddas Ghafarokhy K. Red cell alloimmunization among thalassemic major Chamahal and Bakhtiari province. Congress on Quality Improvement in Clinical Laboratories. Tehran: Laboratory & Diagnosis; 2013.
54. Ghasemi A, Abbasian S, Ghaffari K, Salmanpour Z. Prevalence of alloantibodies and autoantibodies in transfusion dependent thalassemia patients. Iran J Blood Cancer 2016;8:80-5.
55. Esmaeili J. The survey of Rh system and Kell antibody frequency among patients with recurrent blood transfusion. In: 10th National Congress on Quality Improvement in Clinical Laboratory. Tehran: Laboratory & Diagnosis; 2012.
56. Martins PRJ, Alves VM, Pereira GA, Moraes-Souza H. Frequency of irregular antibodies in multiple-transfused patients at the Regional Blood Bank of Uberaba, from 1997 to 2005. Rev Bras Hematol Hemoterapia 2008;30:272-6.
57. Redman M, Regan F, Contreras M. A prospective study of the incidence of red cell allo-immunisation following transfusion. Vox Sang 1996;71:216-20.
58. Santos FW, Magalhães SM, Mota RM, Pitombeira MH. Post-transfusion red cell alloimmunisation in patients with acute disorders and medical emergencies. Rev Bras Hematol Hemoterapia 2007;29:369-72.
59. Dorgalaleh A, Gholami MS, Shokuhiyan M, Valikhani M, Moghaddam ES, Naderi M. Alloimmunization against Rh and Kell blood groups antigens is the main obstacle for blood transfusion in transfusion dependent thalassemia patients in Iran. Int J Case Rep Images 2017;8:358-63.
60. Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion 2003;43:1604-10.
61. Ngoma AM, Mutombo PB, Ikeda K, Nollet KE, Natukunda B, Ohto H. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: A systematic review and meta-analysis. Transfus Apher Sci 2016;54:296-302.
62. Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol 2005;14:143-53.
63. Franchini M, Forni GL, Marano G, Cruciani M, Mengoli C, Pinto V, *et al*. Red blood cell alloimmunisation in transfusion-dependent thalassaemia: A systematic review. Blood Transfus 2019;17:4-15.
64. Verduin EP, Brand A, Schonewille H. Is female sex a risk factor for red blood cell alloimmunization after transfusion? A systematic review. Transfus Med Rev 2012;26:342-53.e1-5.
65. Alves VM, Martins PR, Soares S, Araújo G, Schmidt LC, Costa SS, *et al*. Alloimmunization screening after transfusion of red blood cells in a prospective study. Rev Bras Hematol Hemoterapia 2012;34:206-11.
66. Dogra A, Sidhu M, Kapoor R, Kumar D. Study of red blood cell alloimmunization in multitransfused thalassemic children of Jammu region. Asian J Transfus Sci 2015;9:78-81.
67. Paramore C, Vlahiotis A, Moynihan M, Cappell K, Ramirez-Santiago A. Treatment patterns and costs of transfusion and chelation in commercially-insured and medicaid patients with transfusion-dependent β-thalassemia. Am Soc Hematol 2017;130:5635.
68. Easow Mathew M, Sharma A, Aravindakshan R. Splenectomy for people with thalassaemia major or intermedia. Cochrane Database Syst Rev;2016:6:Cd010517.
69. Evers D, van der Bom JG, Tijmensen J, de Haas M, Middelburg RA, de Vooght K, *et al*. Splenectomy protects humans from red cell alloimmunization. Am Soc Hematol 2016;128(22):24.
70. Samarah F, Srour MA, Yaseen D, Dumaidi K. Frequency of red blood cell alloimmunization in patients with sickle cell disease in palestine. Adv Hematol 2018;2018:5356245.