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REVIEW

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HLA Class II regulation of immune response in sickle cell disease patients: Susceptibility to red blood cell alloimmunization (systematic review and meta-analysis)

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Abstract

Background and Objectives: Sickle cell disease (SCD) patients are commonly treated with red blood cell (RBC) transfusion. Pretransfusion tests commonly involve limited serological antibody testing. RBC alloimmunization to RBC antigens is a frequently encountered complication seen in chronically transfused patients. Genetic factors such as the human leukocyte antigen (HLA) are known to influence and regulate immune responses. HLAs are highly polymorphic and play an essential role in regulating immune responses, including RBC alloimmunization. The aim of this study was to conduct a systematic review and meta-analysis to evaluate the association between HLA Class II allelic polymorphisms with the possible risk of developing RBC alloantibodies.

Materials and Methods: Four databases were systematically searched for relevant studies between the years 2000 and 2021 following the PRISMA guidelines. Four articles met the eligibility and quality criterion, and three alleles, HLA-DRB1*04, HLA-DRB1*15 and HLA-DQB1*03, that were found to be potentially associated with an increased risk in alloantibody formation were included.

Results: The primary outcome measure was alloimmunization by RBC antigen exposure in multiply transfused SCD patients. The total estimate of alloimmunization of the SCD patients was 2.33 (95% CI, 1.58–3.44), demonstrating susceptibility to RBC alloantibody formation. Heterogeneity between the studies was insignificant, suggesting the differences associated with random sampling errors. The results showed that SCD patients carry an increased risk of producing RBC alloantibodies.

Conclusion: A strategy to prevent RBC alloimmunization is genotyping for genetically susceptible SCD patients receiving multiple transfusions. Early identification of genetic variants that can potentially increase the risk of RBC alloimmunization could aid in the screening process and selection of phenotypically matched RBC units.

Keywords

HLA Class II, RBC alloimmunization, sickle cell disease

Highlights

- HLA Class II alleles are associated with susceptibility to alloimmunization in multiply transfused patients with sickle cell disease (SCD).
- The variants HLA-DRB1*04, -DRB1*15 and -DQB1*03 confer an increased risk of producing alloantibodies.
- Early identification of genetic factors affiliated with likely alloantibody development would allow selective use of phenotypically matched red blood cell units in genetically susceptible SCD patients.

INTRODUCTION

Background

Sickle cell disease (SCD) is a hereditary condition affecting haemoglobin and blood flow within the body leading to pain and organ failure [1]. SCD is part of a wider group known as haemoglobinopathies and is most common in people of African ancestry. Transfusion of blood products is utilized as a supportive treatment of haemoglobinopathies to reduce morbidity and mortality [2]. Transfusion therapy can be highly beneficial to patients if the necessary safety precautions are considered. Unfortunately, the consequences of transfusions may still occur despite these precautions. SCD patients are highly susceptible to red blood cell (RBC) alloimmunization as they become reliant on blood transfusions (receiving multiple transfusions within their lifetime) to maintain a relatively healthy life.

Among all transfused recipients, alloimmunization to RBC antigens usually occurs in about 2%–5% [3]. However, approximately 40%–50% of the transfused SCD patients develop RBC alloantibodies in the absence of RBC antigen phenotype matching beyond ABO and RhD matching [4, 5]. Alloimmunization to RBC antigens in SCD patients has a significantly higher rate than other chronically transfused patient populations, potentially due to the differences in RBC antigen expression frequencies between people of different ethnic backgrounds (antigen disparity), high transfusion burden, genetic diversity and immune system considerations etc. [3, 4]. As a result, RBC alloimmunization can complicate the selection of future compatible transfusion units and furthermore, increase the risk of haemolytic transfusion reactions (HTRs), and the development of additional RBC alloantibodies and autoantibodies in subsequent transfusions [6–8].

Significance of RBC alloantibodies in polytransfused SCD patients

The formation of alloantibodies and autoantibodies to RBC antigens has the potential to cause clinically significant complications that promote haemolysis, iron overload and RBC alloimmunization [2, 9]. In SCD patients, the most common alloantibodies formed by multiple transfusions are anti-C and anti-E in the Rh blood group system and anti-K from the Kell blood group system [2]. Therefore, SCD patients are usually transfused with ABO and RhD-compatible, and phenotype matched with anti-C, -E and -K negative RBCs as a preventative measure. Alloimmunization is less prevalent in populations where there is higher phenotypic compatibility between the donor and SCD recipient [10–12]. This is supported by the study conducted by Natukunda et al demonstrating a lower rate of RBC alloimmunisation in the Ugandan SCD population [12].

Role of HLA in alloimmunization

Some studies have demonstrated that alloimmunized SCD patients show a worse survival rate than non-alloimmunized patients [13, 14]. It is not known why some patients become alloimmunized while others do not. This has raised questions about the contributions to the high rate of alloimmunization in SCD populations. However, it is known that the immune response is regulated by genetic factors such as human leukocyte antigen (HLA), having the ability to influence alloimmunization [5, 14, 15]. HLA genes are highly polymorphic and are involved in T-cell-mediated immunity [16]. HLA Class II molecules process and present RBC antigens by antigen-presenting cells (APCs) to T-cell receptors (TCR), which, in turn, activate CD4 helper T-cells. This involves the interaction between T and B cells and the differentiation of B cells into plasma cells. CD4+ regulatory T-cells (Tregs) are immune response modulators; the activation of T-cells triggers Tregs to suppress the activation and proliferation of multiple cell types. A study has found that the Treg suppressive function is reduced in antibody responders in SCD patients [17]. Therefore, resulting in alloimmunization to RBC antigens. It is hypothesized that inheriting certain HLA alleles can predispose patients to RBC alloimmunization (Figure 1) [10].

A relationship between HLA polymorphisms and immune responses to various RBC antigens has been established, and these include Duffy^a [18, 19], Kell [20], Diego [21] and Kidd^a [22]. Noizait-Pirenne et al conducted a study to identify the HLA-DRB1 restriction molecules within the anti-Fy^a and anti-K groups in Caucasians. Their study concluded that HLA-DRB1*04 is the major restriction molecule for Fy^a-derived peptides suggesting that stimulation can cause an individual to produce the antibody [18]. Similarly, Raos et al associated HLA-DR and HLA-DQ polymorphisms with alloimmunization to the Fy^a antigen in a Croatian population [19].

Several studies have associated variants of HLA Class II with RBC alloimmunization and whether the inheritance of HLA Class II

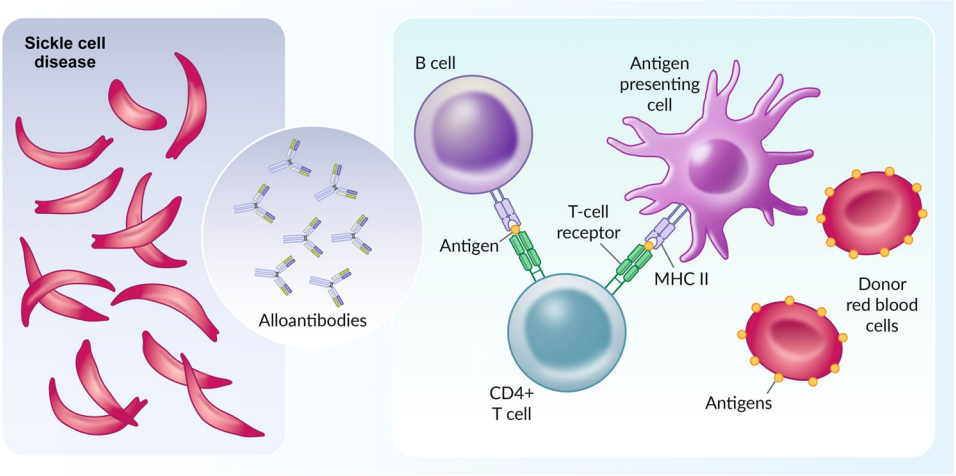

FIGURE 1 Red cell alloimmunization in sickle cell disease patients after exposure to donor cells' antigens

phenotypes provides susceptibility or protection against the formation of RBC alloantibodies [5, 7, 15, 18–26]. However, only a handful of these studies focused on the SCD population [5, 7, 15, 23, 24]. Many studies associated RBC alloimmunization with HLA-DRB1 variants indicating the role of HLA restriction in susceptibility to forming RBC antibodies, particularly in multi-responders. Maluskova et al demonstrated the association of HLA-DRB1*15 with multiple antibody responsiveness in relation to Rh phenotypes [25]. This supported the hypothesis that certain HLA polymorphisms could further increase susceptibility to multi-responsiveness towards RBC antigens.

Scope of review

Alloimmunization or the development of RBC alloantibodies is a major complication of blood transfusion, especially in chronically transfused SCD patients. Susceptibility to alloimmunization is dependent on several different factors, possibly leading to delayed HTRs (DHTR) or other adverse effects. However, not all patients exposed to mismatched RBC antigens become alloimmunized as only a minority of individuals form alloantibodies from exposure [27]. The differences in susceptibility have not yet been fully understood. As SCD patients, homozygous for the disease are multiply transfused, the exposure to different antigens increases the risk of alloimmunization. Some studies have associated HLA-DRB1 with RBC alloimmunization risks. HLA Class II alleles could potentially be a risk factor for developing RBC alloimmunization upon exposure to foreign antigens.

This study evaluates the presence of HLA Class II alleles in alloimmunized and non-alloimmunized SCD individuals homozygous for the sickling phenotype. The primary aim of this study is to investigate the association between HLA phenotype and its correlation to RBC alloimmunization susceptibility within the multiply transfused SCD population. Second, to identify the HLA Class II alleles that can cause an individual with SCD to be more susceptible to forming RBC antibodies after transfusion.

METHODS

Study design

A systematic review and meta-analysis were conducted to investigate the association between HLA Class II polymorphisms resulting in alloimmunization. This study focused on the increased risk of susceptibility to RBC alloimmunization in SCD patients with HLA Class II genetic polymorphisms.

Literature search strategy

For identifying literature for the systematic review and meta-analysis, the study followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines [28]. A systematic search on the PubMed, Scopus, Ovid and Google Scholar databases was conducted between the years 2000–2021, inclusive. The keywords ("HLA" OR "Human leukocyte antigen" OR "MHC") AND ("Sickle cell disease" OR "Sickle cell anaemia" OR "SCD" OR "haemoglobinopathies") AND "Alloimmunization" were used in conjunction during the searches. MeSH terms for variations in "HLA" and "sickle cell disease" were included to broaden the search. Furthermore, a manual search for additional literature was conducted. All articles retrieved underwent an eligibility criterion.

Inclusion and exclusion criteria

The PICO strategy (Population, Intervention, Comparison, Outcome) [29] was adopted for the study selection process to determine a generalized eligibility for inclusion of studies. Population: patients with SCD (homozygous for the HbSS genotype); Intervention: the HLA Class II allelic polymorphisms; Comparison: non-alloimmunized individuals; Outcome: resulting in the alloimmunization of red blood cells (RBCs) confirmed by serological or molecular techniques. The criteria

did not have a restriction on sample size or specific HLA Class II polymorphic gene. However, the inclusion criteria required (a) original articles, (b) the development of RBC alloimmunization associated with HLA polymorphism, and (c) a sample of patients with SCD (homozygous HbSS). Exclusion criteria were all articles that were (a) not in English, (b) duplicates, (c) other systemic reviews, (d) those unrelated to HLA or alloimmunization of RBCs or SCD, and (e) full texts that were unavailable.

Data extraction

The information obtained from the literature included the following information: author, year, sample size, objectives, design characteristic (prospective, retrospective), patient population, country of study implementation, HLA allele identified and methods used. Each study was assessed using a STROBE (Strengthening the Reporting Observational studies in Epidemiology) checklist [30] to investigate the suitability for the meta-analysis.

Statistical analysis

Data collected from the studies compared the number of alloimmunized and non-alloimmunized SCD patients with the presence of HLA-DRB1*04, HLA-DRB1*15 or HLA-DQB1*03. The association between increased susceptibility to alloimmunization and HLA Class II polymorphisms was assessed using odds ratios (ORs) with 95% confidence intervals (95% CI).

From the selected studies, the binary random-effect method utilizing the maximum likelihood model approach was selected to estimate the effect size as an odds ratio with 95% CI and heterogeneity. Significance was determined using p-value (where $p \leq 0.05$ is considered statistically significant). The heterogeneity of the studies using the chi-square-based Q test and I^2 test relative to the degree of freedom was evaluated (where $p \leq 0.05$ is considered statistically significant). Thresholds for the interpretation of heterogeneity follows: (i) 0%–40% might not be important, (ii) 30%–60% may represent moderate heterogeneity, (iii) 50%–90% may represent substantial heterogeneity, and (iv) 75%–100% considerable heterogeneity [31].

All statistical analyses were conducted using the Review Manager (RevMan) software (Version 5.4. The Cochrane Collaboration, 2020.) to evaluate the association between SCD patients and HLA alleles relative to RBC alloimmunization.

RESULTS

Study selection and characteristics of the included studies

Between the years 2000 and 2021, a total of 866 articles were collected from the four databases, with an additional 17 articles manually found. Of these 883 articles, 850 articles were excluded, as described in Figure 2. The remaining 33 articles were eligible for the systematic review. Among the 33 eligible articles, only four of these presented an analysis of HLA Class II alleles in SCD patients causing susceptibility towards alloimmunization and were included in the meta-analysis.

The four eligible studies presented analyses of HLA-DRB1 and/or HLA-DQB1 alleles within the SCD population. Sufficient data on HLA-DRB1 analysis in association with generalized RBC alloimmunization could be found in three studies [7, 23, 24], and one study [15] contained data on HLA-DQB1 in association with alloimmunization susceptibility. Table 1 summarizes the four studies included in the systematic review and meta-analysis. All studies were either conducted in the USA ($n = 2$) or Brazil ($n = 2$), consisting of all SCD patients with the homozygous genotype. All patients had a history of transfusion, and data on alloimmunization and non-alloimmunization were collected by each study. The studies varied in the HLA Class II allele found to be associated with alloimmunization susceptibility between HLA-DRB1*15, HLA-DRB1*04 and HLA-DQB1*03. The study conducted by Rodrigues et al [15], associated HLA-DRB1*04 and HLA-DRB1*11 to susceptibility but more specificity to producing anti-Fy^a and anti-K antibodies. HLA detection was consistent among all studies using polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO). The method of alloimmunization detection varied; however, the primary endpoint was any RBC alloimmunization in all cases.

Quality assessment of studies

The quality of the four eligible studies was analysed and assessed using the STROBE standards for the meta-analysis. Table 2 addresses the most important points within each area of the studies conducted, according to the author. The following criterion included were determined from (1) introduction, (2) methods, (3) results, and (4) discussion. Within these four criterion areas, the quality of the studies was based on (1a) rationale explained, (1b) objectives specified with a hypothesis, (2a) patient eligibility provided, (2b) outcomes and potential confounders defined, (2c) bias addressed, (2d) describes all statistical methods, (3a) number of individuals at each stage reported, (3b) give adjusted estimates, and (4a) states limitations. The quality of each study for criteria was assessed as either "yes" or "no".

Patient eligibility was reported in three of the four studies, describing the sample size and number of transfusion histories required for data inclusion. The fourth study [7], however, did not clearly state the patient eligibility but did state the number of both alloimmunized and non-alloimmunized patients. In assessing the studies, only the study conducted by Sippert et al contained a healthy control group of experimental SCD patients. These healthy control patients with a similar ethnic background to the experimental groups served as the basis for allele frequency of polymorphism determination. One study [23] failed to define potential confounders in the study. Another study [23] failed to state any limitations. Overall, the bias of the primary studies was not well addressed. The transfusion histories of the patients for the studies were obtained from

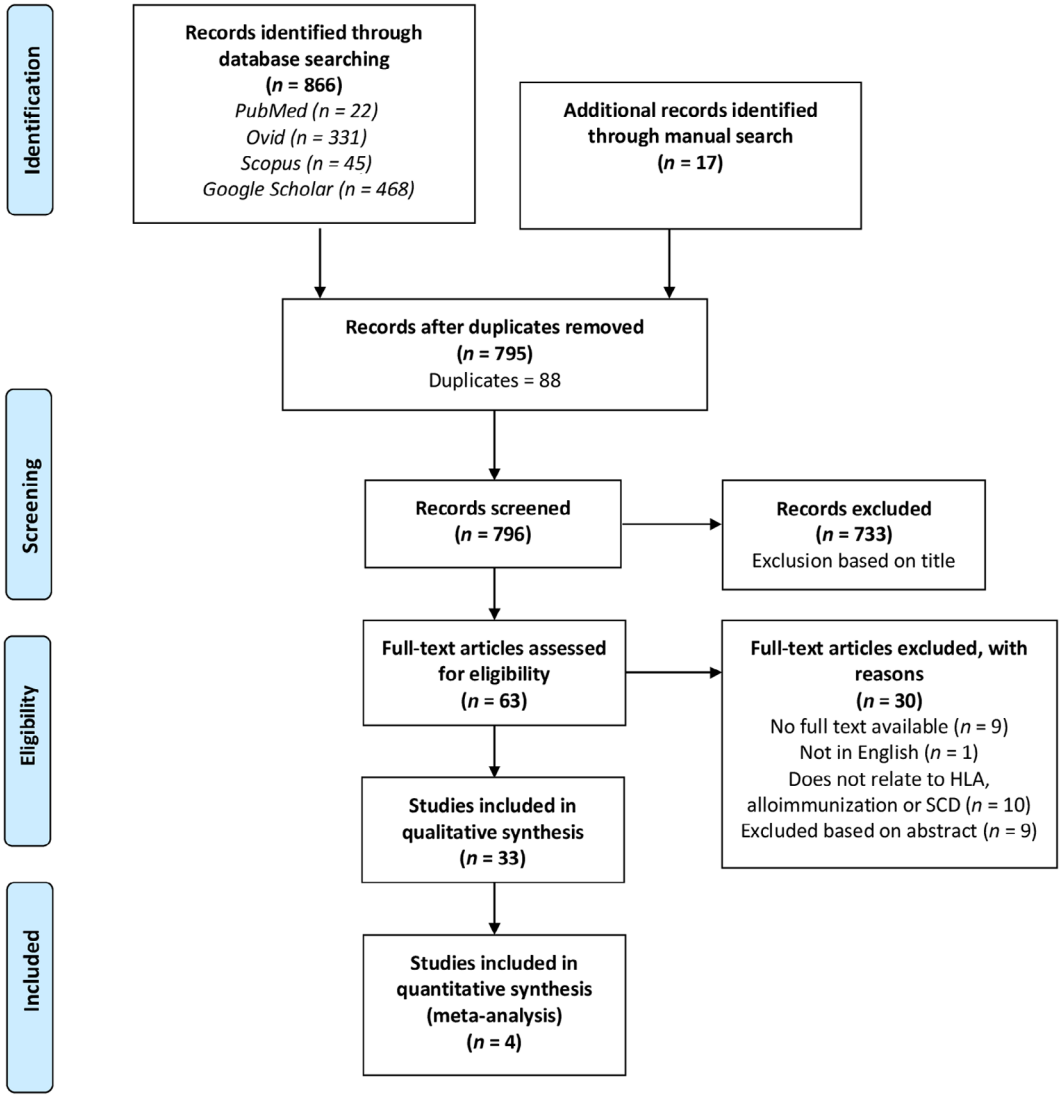

FIGURE 2 Flow diagram summary of a study selection process involving four databases (PubMed, Ovid, Scopus and Google scholar). A manual search was conducted in conjunction with 88 article duplications removed. Four major steps in the study selection process involve the general identification of articles, a screening process to exclude articles based on titles, followed by an eligibility check and finally, studies to be included in the meta-analysis based on relevant quantitative data [28]

institutional blood and medical records [7]; the University of Campinas (UNICAMP) Haematology Blood Center and Maringa Regional Blood Center [15]; the Hematology and Hemotherapy Center of the UNICAMP [23]; and the Children's National Medical Center [24].

Data analysis association

All included studies demonstrated that SCD patients have an increased risk of alloimmunization compared to the control group. The pooled results indicated a statistically significant association (OR = 2.33; 95% CI, 1.58–3.44; $p \leq 0.0001$). The heterogeneity between the studies was not important ($I^2 = 0\%$; $p = 0.88$) (Figure 3).

Table 3 summarizes the results from the four studies of SCD patients associated with increased susceptibility to RBC antibody production. The frequency of the alloimmunized SCD patients is greater than the non-alloimmunized patients. Figure 4 summarizes the frequency of HLA alleles that is associated with increased susceptibility to RBC antibody production in each of the included studies; however, the overall effect was not statistically significant (OR = 1.32; 95% CI, 0.76–2.30; $p = 0.32$).

Analysis of HLA Class II and alloimmunization/ strength of association

The majority of the studies addressing HLA polymorphism in association with generalized RBC alloimmunization reported on HLA-DRB1 alleles. Common antibodies reported in the studied alloimmunized patients included antibodies developed against the Rh system (anti-E,

TABLE 1 Summary of studies included in the meta-analysis on HLA Class II associated with RBC alloimmunization in SCD patients

Study	Study design	Sample size	Number of alloantibody positive & negative	Country	Population (nationality)	Method of HLA detection	Method of alloimmunization detection	HLA Class II associated with susceptibility
Hoppe C. [7]	Retrospective	159	+ve = 59 −ve = 100	USA	SCD (HbSS mutation) – American	PCR-SSO(Probe)	Standard (gel and antiglobulin techniques)	HLA-DRB1*15 & HLA-DRB1-11, HLA-DRB1*13
Rodrigues C. [15]	Prospective	172	+ve = 44 −ve = 128	Brazil	SCD (HbSS mutation) – Brazilian (mixed ethnic group)	PCR-SSO	Blood group genotyping by DNA microarray	HLA-DQB1*03 & HLA-DRB1*04, HLA-DRB1*11
Sippert E. [23]	Prospective	161	+ve = 67 −ve = 94	Brazil	SCD (HbSS mutation) -Brazilian	PCR-SSO	Serologic and molecular (Immucor BioArray Beadchip)	HLA-DRB1*15
Tatari-Calderone Z. [24]	Retrospective	204	+ve = 88 −ve = 116	USA	SCD (HbSS mutation) – African American	PCR-SSO using IMGT database	N/A	HLA-DRB*04

Abbreviations: +ve, positive; −ve, negative; HbSS, haemoglobin SS; SCD, sickle cell disease.

TABLE 2 STROBE checklist for studies used in meta-analysis identified across the different areas of a study. Using a series of criteria to assess the relevance and quality of studies

	Introduction		Methods				Results		Discussion
Study	Rationale explained	Objectives specified with hypothesis	Patient eligibility provided	Outcomes, and potential confounders defined	Bias addressed	Describes all statistical methods	Number of individuals at each stage reported	Give adjusted estimates (95% CI)	States limitations
Hoppe C. [7]	Y	Y	N	N	Y	Y	Y	Y	Y
Rodrigues C. [15]	Y	Y	Y	Y	N	Y	Y	Y	N
Sippert E. [23]	Y	Y	Y	N	N	Y	Y	Y	Y
Tatari-Calderone Z. [24]	Y	Y	Y	Y	Y	Y	Y	Y	Y

Abbreviations: 95% CI, 95% confidence interval; N, No; Y, Yes.

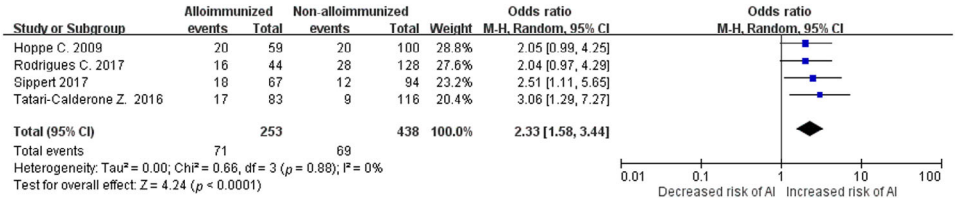

FIGURE 3 Forest plot of the risk of alloimmunization in SCD patients. Results were expressed as the odds ratio (OR) with 95% confidence intervals (CI), the random-effect model (DerSimonian and Laird method) was used for the overall effect, and mantel–Haenszel method (M-H) was used for the heterogeneity. Statistical significance of the forest plot was expressed as Z-score and *p*-value; heterogeneity was described as I^2 with a different *p*-value. The weight was defined as % regarding the size of each study

TABLE 3 Summary of studies reporting number and frequency of alloimmunized and non-alloimmunized SCD patients

Study	Red cell units chosen for the patients	Number of alloimmunized/total alloimmunized	Alloimmunized frequency (%)	Number of non-alloimmunized/total alloimmunized	Non-alloimmunized frequency (%)	OR (95% CI)
Hoppe C. [7]	At least Rh and Kell matched	20/59	34.00	20/100	20.00	2.05 (0.99, 4.25)
Rodrigues C. [15]	At least Rh and Kell matched	31/44	35.23	55/128	21.48	2.04 (0.97, 4.29)
Sippert E. [23]	At least Rh and Kell matched	18/67	13.85	12/94	6.98	2.51 (1.12, 5.65)
Tatari-Calderone Z. [24]	At least Rh and Kell matched	17/88	19.31	9/116	7.76	2.85 (1.20, 6.74)

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.

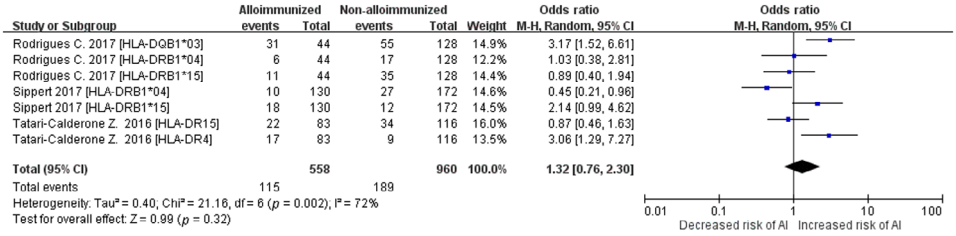

FIGURE 4 Forest plot of the frequency of HLA alleles that are associated with increased susceptibility to RBC antibody production in each included study. Results were expressed as the odds ratio (OR) with 95% confidence intervals (CI), the random-effect model (DerSimonian and Laird method) was used for the overall effect, and mantel–Haenszel method (M-H) was used for the heterogeneity. Statistical significance of the forest plot was expressed as Z-score and *p*-value; heterogeneity was described as I^2 with a different *p*-value. The weight was defined as % regarding the size of each study

TABLE 4 Strength of association of HLA Class II alleles to alloimmunization rate

Study	HLA phenotype	RBC antibody	Alloimmunized (ab present)	Non-alloimmunized (ab absent)
Hoppe C. [7]	HLA-DRB1*04 HLA-DRB1*11	Anti-Fy[a] Anti-K	100% 47%	12% 19%
Rodrigues C. [15]	HLA-DRB1*11 & -DRB1*13	Anti-K	70%	50%
Sippert E. [23]	HLA*DRB1*15	Rh system (D,C,c,E,e)	15.63%	6.98%
Tatari-Calderone Z. [24]	HLA-DRB1*04	Anti-Fy[a]	100%	19.01%

Abbreviations: Ab, antibody; HLA, human leukocyte antigen; RBC, red blood cell.

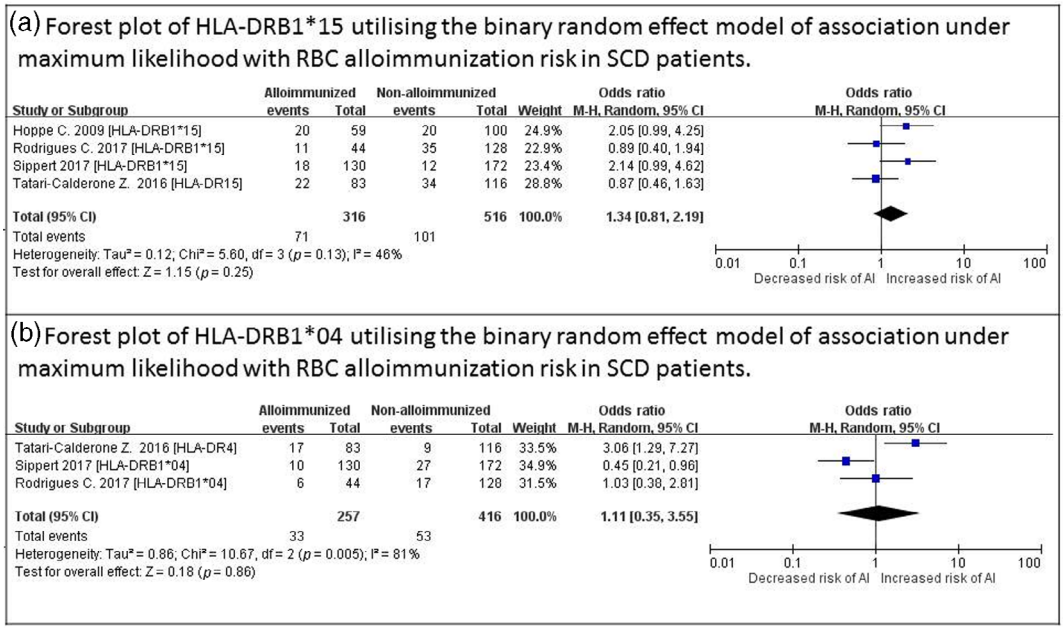

FIGURE 5 (a) Forest plot of HLA-DRB1*15 utilizing the binary random-effect model of association under maximum likelihood with RBC alloimmunization risk in SCD patients. (b) Forest plot of HLA-DRB1*15 utilizing the binary random-effect model of association under maximum likelihood with RBC alloimmunization risk in SCD patients. Results were expressed as the odds ratio (OR) with 95% confidence intervals (CI), the random-effect model (DerSimonian and Laird method) was used for the overall effect, and mantel–Haenszel method (M-H) was used for the heterogeneity. Statistical significance of the forest plot was expressed as Z-score and *p*-value; heterogeneity was described as I^2 with a different *p*-value. The weight was defined as % regarding the size of each study

anti-C, anti-D, anti-C^w, anti-c and anti-e) and the Kell system (anti-K). Other antibodies less frequently reported included anti-Fy^a, anti-S, anti-Jk^b, anti-Le^a, anti-M, anti-I and more [7, 15, 23, 24].

The studies included have associated some HLA Class II alleles with specific alloantibody formation as seen in Table 4. In the study conducted by Rodrigues et al, an increased prevalence of HLA-DRB1*04 was observed in anti-Fy^a patients (100%) in comparison to the alloantibody negative control patients (12%). Rodrigues et al have also demonstrated the predominance of the HLA-DRB1*11 allele in patients with anti-K (47%) compared to those without anti-K (19%) [15]. Similarly, Sippert et al reported a prevalence of the HLA-DRB1*15 allele in patients alloimmunized to the Rh system antigens (D, C, E, c, e) (15.63%), compared to the non-alloimmunized controls (6.98%) [23]. Furthermore, Hoppe et al found an excess of HLA-DRB1*11 and -DRB1*13 alleles in anti-K patients (70%) when compared to the controls (50%) [7]. Tatari-Calderone et al found that the HLA-DRB1*04 molecule acts as a restriction molecule in the development of anti-Fy^a antibodies in the Caucasian population. They demonstrated that 100% of individuals expressing the -DRB1*04 phenotype with anti-Fy^a compared to 19.01% non-alloimmunized individuals carrying the -DRB1*04 phenotype. However, Tatari-Calderone et al, reported a conflicting statement to Rodrigues and coworkers that HLA-DQB1*03 conveyed a protective role towards RBC alloimmunization [24].

Four studies were included in the meta-analysis for the association between HLA-DRB1*15. However, due to the varying results from each study, the result was not statistically significant (OR, 1.34; 95% CI, 0.81–2.19; $I^2 = 46\%$; $p = 0.25$) as demonstrated in Figure 5a. Moreover, three studies were included in the meta-analysis for the association between HLA DRB1*04, but the result was not statistically significant due to the limited number of studies included and the result variation (OR, 1.11; 95% CI, 0.35–3.55; $I^2 = 81\%$; $p = 0.86$) as illustrated in Figure 5b.

DISCUSSION

SCD individuals require many transfusions in their lifetimes for related complications to the sickling disease. Factors increasing the risk of RBC alloimmunization in SCD patients remains largely unclear, however, genetic factors are known to influence immune regulatory responses. The risk of alloimmunization is related to the number of red cell transfusions (transfusion intensity) and the degree of prophylactic antigen matching. Alloimmunization is dependent on the presentation of donor RBC-related peptide antigens by APCs to the recipient's T-cell receptors. Therefore, HLA Class II molecules are essential for RBC alloimmunization. A decrease in alloimmunization rates has been shown in SCD patients transfused with RBCs matched for C/c, E/e and K antigens in multiple single-group studies by the ASH 2020 guidelines for SCD transfusion support [32]. This demonstrated that SCD patients receiving Rh and K-matched red cells showed an alloimmunization risk of 18% (95% CI, 10%–27%) acquired from 15 studies. However, despite prophylactic measures to ensure more compatible units are provided, RBC alloimmunization remains

highly prevalent in multiply transfused SCD patients. Many SCD individuals are still developing antibodies to the Rh system antigens despite the serological matching of units. This may be due to allelic polymorphisms within the blood group systems, mainly the Rh system, off-protocol transfusion, immune hyper-responsiveness during proinflammatory events or other unidentified causes [33].

The current systemic review and meta-analysis examined the association of HLA polymorphisms and RBC alloimmunization in SCD patients from Brazil and the United States. The review and meta-analysis have confirmed that the genetic inheritance of some HLA alleles predisposes individuals to RBC alloimmunization. These observations led to the findings that antibody production is not only caused by chronic transfusions but also by genetic components influencing the development of alloantibodies [34].

Although all studies selected the minimum Rh and Kell matched RBC units for the patients, most of the antibodies identified in the alloimmunized groups were in the Rh and Kell systems. While some alloimmunization may be due to polymorphisms in the RH and Kell system genes, it is likely that prophylactic matching was incompletely practised. Between the articles used in this review, there are some contradictions in relation to which alleles cause alloimmunization susceptibility and to which blood group antigens.

Interpretations

The overall result of the present study indicates the odds of SCD patients developing RBC alloantibodies are greater than non-SCD patients after antigenic exposure. An OR of 2.33 demonstrates statistically significant evidence that there is an increased odds of developing alloantibodies if an individual is an SCD patient. The heterogeneity demonstrated no importance and thus, indicates that the differences between the studies are due to random sampling error. This assures that genetic inheritance of HLA Class II alleles producing alloimmunization risk will cause similar effects in other multiply transfused populations. These results are supported by studies relating HLA Class II alleles, mainly HLA-DRB1 polymorphisms, to specific alloantibody development in the polytransfused population.

The primary studies from this review identified associations of HLA Class II alleles with anti-Fy^a, anti-K and antibodies to the Rh system antigens. Evidence towards alloimmunization with anti-Fy^a showed a strong relationship with the HLA-DRB1*04 allele [15, 24]. The majority of SCD patients are of African descent, where many have the Fy(a-b-) phenotype mostly due to the GATA mutation [35]. Although these individuals are serologically negative for Fy^b on their RBCs, other cells will, however, express Fy^b. This means that there is a low risk of developing an antibody to the Fy^b antigen [35]. As such, it possibly explains why alloimmunization risk is not associated with anti-Fy^b. Furthermore, a moderately strong association with anti-K alloimmunization was found with mainly HLA-DRB1*11; however, antibodies to the Rh system antigens were not as convincing when associated with HLA-DRB1*15.

Many other studies have investigated the role HLA alleles play in alloimmunization susceptibility of other populations, which reinforces the findings of this current review. In a study by Reviron et al [22], HLA-DRB1*01 was found significantly more frequent in patients with anti-Jk^a than the controls, 55%–17%, respectively [22]. Similarly, Baleotti et al [21] had concluded that there was an overrepresentation of HLA-DRB1*07 in patients alloimmunized with anti-Di^a compared to non-alloimmunized patients, 75% and 27%, respectively. Furthermore, HLA-DRB1*11 demonstrated by Chiaroni et al [20] associated with K immunization was significantly higher in alloimmunized patients compared to controls (57% vs. 28%, respectively); this was confirmed by Rodrigues et al. In confliction to Chiaroni and colleagues and Rodrigues and colleagues, Noizat-Pirenne et al [18] identified several HLA-DRB1 groups that were seen increasingly in anti-K individuals. Furthermore, HLA-DRB1*15 has been shown to be involved with alloimmunization by the D and Fy^a antigens [19, 23, 36]. Raos et al and Picard et al had also found an overrepresentation of HLA-DRB1*04 and/or HLA-DRB1*15 alleles in anti-Fy^a patients in the Croatian and Southern European population, respectively (96% for both) [19, 37].

Due to linkage disequilibrium between alleles across the major histocompatibility complex (MHC), it is difficult to determine the direct response that an HLA phenotype has in association with RBC alloimmunization [23]. Despite this, the results have demonstrated that HLA Class II alleles convey susceptibility to RBC alloimmunization, especially HLA-DRB1, supported by several studies across different populations.

Implications to clinical practice

Due to the presence of alloantibodies in chronically transfused SCD patients that cause HTRs and other transfusion-related complications making it difficult to obtain compatible blood units, it is important to investigate and identify the association between HLA and alloimmunization. This will allow for the best selection of compatible, phenotype-matched units for patients. Rodrigues and colleagues [15] investigated the possibility of molecular techniques in routine laboratory immunohaematology to increase the safety and efficacy of transfusion. SCD is most commonly found in patients of African descent, showing a high rate of complex Rh antibody variants and antibodies to low-incident antigens [38]. Hendrickson and colleagues [37] concluded that antigen matching based on phenotype alone between African American donors to African American SCD recipients may not decrease alloimmunization rates to complex Rh antibody variants. Therefore, making genotyping RBC antigens for matched units with a beneficial alternative.

Overall, a decrease in alloimmunization rates among sickle cell patients has been demonstrated with the use of genotyping. A Brazilian study found that 11 of 15 alloimmunized patients benefited from receiving RBC units based on genotype [39]. However, due to limited blood supply, molecular matching may not be a feasible option as it is also a costly procedure. Such a precise matching practice

makes routine transfusion difficult for both the transfusion service and donor centre and is, therefore, not widely applied.

Strengths and limitations

This present review systematically examines the association between HLA Class II and RBC alloimmunization with respect to HLA-DRB1 and -DQB1 alleles. Despite the many ways to attain alloimmunization, these factors did not seem to affect the results. The review defines a more specific subgroup of chronically transfused patients, SCD individuals.

However, this systematic review is unable to conclude the association between specific alleles and the risk of alloimmunization due to the limited number of studies incorporated. The included studies show different HLA associations, and the overall results are not statistically significant (Figure 5a,b).

Future studies should include a larger sample size with a healthy control group. More extensive investigation in SCD patients would need to be conducted as there are still some discrepancies between the alleles conveying susceptibility. Thus far, convincing evidence towards HLA-DRB1*04 resulting in anti-Fy^a has been illustrated. However, more is required to identify which SCD patients are responders or non-responders based on homozygosity, heterozygosity or other factors that may play a role.

This study could not account for other factors, including age, gender, and the number of previous transfusions, which may impact results. Additionally, homozygosity and heterozygosity of alleles were not considered, such as a haplotype with alleles considered as protective and susceptible. Moreover, the studies did not directly compare the difference between serologic and genotypic matching for the prevention of RBC alloimmunization. All primary studies determined the alloimmunization outcome; however, not all studies assessed the risk of bias. Rodrigues et al and Sippert et al corrected the data with Bonferroni's method [15, 23].

It is not well known why SCD patients given antigen-negative units could develop new alloantibodies. A further study focusing on the association between HLA and RBC alloimmunization in SCD patients will be highly beneficial for healthcare professionals. It could provide clinicians and transfusion services with a better understanding of the risk of multiple transfusions in SCD patients and introduce a suitable strategy to prevent the development of alloantibodies.

CONCLUSION

There are routine screening and crossmatching of blood in place to identify and prevent as many incompatibilities as possible. However, due to the requirement for more than one transfusion and the lack of antigen-negative units, there is a high risk for SCD patients to become alloimmunized. In conclusion, the result from this study suggests that there is no statistically significant evidence indicating that HLA Class II alleles are associated with susceptibility to alloimmunization in multiply transfused SCD patients. The reasons are unknown as to how HLA alleles impact alloimmunization susceptibility. Further investigation should be conducted into the GATA mutation and whether there is a susceptibility or risk factor towards RBC alloimmunization. Furthermore, while genetic variants are not picked up in routine screening, further study is needed into the use of genotyping for extended matching in SCD patients as a preventative measure to alloantibody formation. Heterozygosity and/or homozygosity inheritance of the alleles should be investigated for the same susceptibility effects. This knowledge could help change the screening process for HLA in SCD patients to better the transfusion system. Early identification of genetic factors affiliated with likely alloantibody development would allow for selective use of phenotypically matched RBC units in genetically susceptible SCD patients. This could ultimately reduce the likelihood of RBC alloimmunization.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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