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ORIGINAL ARTICLE

Serological and Molecular Compatibility Methods Among Sickle Cell Disease Blood Recipients and Their Donors in a Tertiary Hospital, in North Central Nigeria: a Comparative Study

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ABSTRACT

Introduction: Sickle Cell Disease (SCD) is an autosomal recessive genetic blood disorder characterized by abnormal, rigid, sickle cell shape in a hypoxic environment. Most SCD patients require a safe and compatible blood transfusion during crises; hence, there is a need for DNA genotyping blood to avoid haemagglutination in multiple transfused patients. This study compared molecular genotyping to serological compatibility testing methods among sickle cell disease patients attending General Hospital Ilorin.

Materials and Methods: Fifty sickle cell disease recipients and fifty blood donors in General Hospital Ilorin were randomly selected. Five milliliters of blood samples were collected from each group. Serological compatibility testing was performed on each sample using standard tube agglutination methods. Molecular compatibility testing was done by extracting the genomic DNA, followed by polymerase chain reaction techniques. The products were separated by Gel electrophoresis; results were interpreted and analyzed.

Results: The Mean age of the Sickle cell disease recipients is 7.6 (± 4.3) years. Their Mean Body weight is 23.6 (± 8.2) Kg. About 30 (60%) of the SCD patients have been transfused at least twice before, with 50 (16%) history of blood transfusion reaction. Most recipients (52%) are of O Rh D positive blood group while the donors are (56%). All the recipients had a negative Indirect Antiglobulin Testing, with 100% compatible for serological and 80% for Molecular procedures. There are ten discrepancies between serological phenotyping and molecular RHCDE genotyping. Four were due to Rh D antigens, four were also due to RhCE antigens and two were due to both RhCDE antigens. However, there was complete compatibility between serological and molecular compatibility of forty sickle cell disease.

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Conclusion: Molecular compatibility testing offers superior sensitivity, specificity, and accuracy compared to serological compatibility testing methods and should be considered the gold standard when transfusing sickle cell disease patients.

Keywords: *'Serological compatibility', 'Molecular compatibility', 'Sickle cell disease'.*

INTRODUCTION

Sickle Cell Disease (SCD) is an autosomal recessive genetic blood disorder characterized by red cells which transform into abnormal, rigid, sickle-shaped red RBCs, when they are in a hypoxic environment (1). SCD may lead to various acute complications with a high mortality rate, such as splenic sequestration crisis, aplastic crisis, and haemolytic crisis (2). Blood transfusion with normal haemoglobin A red blood cell (RBC) increases oxygen saturation and reduces red blood cell sickling. Therefore, blood transfusion is the practical and standard management of acute complications of SCD (3). Most SCD patients require a compatible blood transfusion during crises. Hence, there is a need for DNA genotyping blood to avoid haemagglutination in multiply transfused patients. Furthermore, chronic RBC transfusion therapy is efficacious in reducing the risk of complications and silent stroke in children with SCD (4). These patients may need life-long RBC transfusions to alleviate the chronic anaemia and further suppress extramedullary haematopoiesis.

One of the significant side effects of chronic blood transfusion therapy is alloimmunization to foreign RBCs. The incidence of RBC alloimmunization in the general population is approximately 2% to 6%. However, it may be as high as 36% in patients with SCD (1). Alloimmunization is the source of a variety of problems during long-term RBC transfusion management and is associated with an increased incidence of delayed haemolytic transfusion reactions and a reduction in available compatible blood for future transfusion in subsequent crises (5,6).

There are numerous blood group systems, among which ABO and Rh blood groups contain the antigens provoking the most severe transfusion reactions (7,8). Whenever possible, these chronic blood transfusion recipients whose RBCs lack those antigens should receive compatible Ag-negative RBCs to avoid the generation of corresponding antibodies (9). Therefore, accurately identifying the antigens on recipient RBCs is critical in preventing RBC alloimmunisation (10). However, due to the presence of donor RBCs in the recipients' circulation, which may persist for months, accurate Ag typing of these transfused recipients by traditional serological methods is often difficult, if not impossible. Furthermore, antigen typing is more complicated when the recipient's RBCs have a positive direct antiglobulin test. Thus, molecular genotyping is needed.

Blood transfusion involves pre-transfusion testing, which involves crossmatching donor blood of appropriate ABO and Rh D type for a patient requiring it. The donor blood is considered compatible if no observable reaction occurs between the donor and patient blood. The most important steps during this process are correct patient identification and selecting blood of the correct ABO group for the patient. Cross-matching is a simple process, but the wrong blood may be transfused if not carried out correctly, leading to disastrous

consequences. Karl Landsteiner, a pioneer in the field of transfusion, played a significant role in the development of the ABO blood group system. This system differentiates red blood cells based on their surface antigen structures and provides blood that survives for the maximum period. Despite the existence of over three hundred genetically different blood groups, the ABO and Rh blood group system remains the most clinically important in transfusions. This advancement in genetic methods has significantly improved the quality of blood transfusion services, ensuring patients receive blood that survives for the maximum period possible. (10)

The ABO classification is a standard procedure in clinical laboratories to determine blood groups, including A, B, AB, O, and Rh. However, unexpected antigens or other blood group antigens may occur in individuals without specific red blood cell antigens, leading to transfusion reactions. To ensure successful and safe blood transfusion, it is crucial to know the compatibility of donor and recipient blood group systems, such as ABO, Rh, K, fya, fyb, jka, jkb, and MNS. Incompatible or mismatched transfusions can lead to severe consequences and sudden death. Cross-matching tests between the intended donor and patient are highly recommended for routine clinical analysis. Hemagglutination has been the gold standard for red blood cell (RBC) antigen detection for over a century, allowing the terms "serology" and "immunohematology" to become familiar. Despite its low cost, ease of performance, sensitivity, and specificity, hemagglutination-based determination of RBC phenotype has limitations. Nucleic acid-based technologies have been added to advanced immunohematology reference laboratories to mitigate these limitations and allow the term "molecular immunohematology" to enter the blood banking lexicon.

Molecular immunohematology uses genotyping to predict an individual's RBC blood group phenotype, utilizing genes encoding antigens. Initially considered complex, costly, and challenging to automate, genotyping is now a valuable complement to hemagglutination. Advances in understanding the relationship between RBC genotype and phenotype, detailed descriptions of blood group genes and silencing elements, and nano and chip technology have made genotyping a valuable tool for detecting red blood cell nucleotide polymorphisms. Thus, transfusion authorities are now considering molecular techniques in transfusion medicine as critical to advancing the understanding of blood group antigen polymorphisms and increasing blood safety by providing better-matched, compatible homologous blood products for transfusion (11).

A recent study suggested molecular compatibility testing in blood banks to resolve some of the apparent inconsistencies of hemagglutination on transfusion. Hence, this study aims to compare molecular genotyping as applied to serological compatibility testing methods among sickle cell disease patients attending a tertiary hospital in Ilorin and the apparent path to a stepwise implementation and adoption plan for this important and emerging technology.

RBC transfusions are typically performed for ABO and D antigens, but there are instances where multiple antigen-negative RBC products are requested, especially for patients with sickle cell disease (SCD). Alloimmunization occurs in 2% to 6% of patients receiving RBC transfusions, but rates can be as high as 36% in SCD patients (12). Recipients with multiple alloantibodies can hinder the ability to provide antigen-negative, compatible RBCs, as blood banks must often phenotype to find an appropriate product. This burden on donor centers includes time, adequate RBC inventory, and appropriate reagents. Mass-scale genotyping aims to expand phenotype/genotype matching for a larger patient population, improving patient care. Phenotype-matched products benefit patients chronically transfused or at increased risk for alloantibody formation. These products can

be limited to C, E, and K antigens or extended to include Fya, Jka, Jkb, S, and other antigens (13). The primary reason for phenotype-match products is to prevent alloantibody formation and adverse haemolytic transfusion reactions (14).

Transfusion with phenotypically matched RBC units, specifically for the C, E, and K antigens, can reduce the risk of alloimmunization in patients with sickle cell anaemia. Institutions often provide these units, but prophylactic matching for additional antigens should be considered once a patient develops an antibody (6,15). Donor centres use phenotype-matching to screen and stock RBC products for transfusion services, but this practice is time-intensive and expensive. The transfusion service uses algorithms to determine the likelihood of an antigen-negative product based on limited phenotyping and donor race and ethnicity. Still, batch serologic screening is time-consuming and requires appropriate controls. This practice is limited to specific patient populations and does not apply to all donor centres.

HLA testing is a crucial tool in identifying and diagnosing SCD, a disease characterized by the presence of a specific virus. This testing involves placing a particular type of virus, such as HLA, by examining the red blood cells (RBC) of already phenotyped patients and receiving phenotype-matched RBCs. This stepwise approach to transfusion services can improve RBC matching and patient outcomes. DNA-based testing for RBC antigens provides an alternative typing method that is not subject to the limitations of serologic typing (16). The molecular basis of blood group antigens is utilized for antigen typing in blood donors and patients. Unlike serologic typing, DNA-based tests detect genes encoding RBC antigens. Clinically significant antigens are encoded by alleles defined by single nucleotide polymorphisms (SNPs), allowing for the prediction of probable phenotype. SNP assays detect the common clinically significant antigens, C/c, E/e, K/k, Fya /Fyb, Jka /Jkb, M/N, and S/s (13). Semi-automated platforms allow testing for multiple SNPs in a single assay. Such assays have been applied to the mass screening of blood donors for desirable antigen-negative combinations or the absence of high-prevalence antigens. Their use in the setting of the hospital transfusion laboratory has been relatively limited.

Recently transfused patients who are transfusion dependent, such as those with SCD, thalassemia, and aplastic anaemia, are challenging to phenotype when no pre-transfusion sample is available. Because of the presence of circulating donor RBCs, which may persist for weeks, determining an individual's phenotype by traditional hemagglutination methods is complex due to the presence of a mixed-field population (17). Using time-consuming and labour-intensive techniques, such as isolating reticulocytes or sickle cells (in individuals with SCD), the RBC phenotype may be determined but may be inaccurate. Molecular methods can overcome this limitation of hemagglutination (18). Because transfusion does not affect somatic cells, using buccal epithelial cells and urine sediment as a source of DNA can provide accurate results (19). It is also possible to obtain DNA from white blood cells in the peripheral blood (20); however, some have criticized that interference from circulating donor white blood cells may affect the result. In addition, RBC genotype determination is superior to traditional hemagglutination techniques for patients with circulating autoantibodies, including AIHA. IgG removal from RBCs can prevent false-positive antigen typing, especially in warm autoantibodies (20). However, many RBC typing reagents now contain monoclonal antibodies, allowing for direct agglutination phase antigen detection. In cases where direct agglutinating reagents are unavailable, the antigen is sensitive to IgG removal treatment, or IgG removal is ineffective; genotyping provides an acceptable alternative by predicting a patient's

RBC antigen status without autoantibody interference. In summary, determining a patient's RBC genotype is superior to traditional hemagglutination techniques.

In terms of comparison of the Molecular and the serological testing methods, a study that applied genotyping to chronically transfused patients with SCD found discrepancies in 6 of 40 patients between the serologic RBC phenotype and phenotype predicted by the genotype in the patients with SCD but not the control group. The serologic phenotype mistypes were secondary to recent transfusion. The six patients who then were switched to the correct antigen-matched RBCs had improved RBC survival with diminished frequency of transfusions. This study aims to compare the use of genotyping patients with SCD for antigen-matched RBC transfusions with serological phenotyping.

Blood transfusion is the practical and standard management of acute complications of SCD (21). Furthermore, chronic RBC transfusion therapy is efficacious in reducing Chronic anaemia in these patients and may also cause extramedullary haematopoiesis, which can lead to bone deformities. These patients may need life-long RBC transfusions to alleviate the chronic anaemia and further suppress extramedullary haematopoiesis. One of the significant side effects of serological compatibility testing is alloimmunization (22). The incidence of RBC alloimmunization in the general population is approximately 2% to 6%. However, it may be as high as 36% in patients with SCD (12). Alloimmunization is the source of various problems during long-term RBC transfusion management and is associated with an increased incidence of delayed haemolytic transfusion reactions and a reduction in available compatible blood for future transfusion in subsequent crises. Accurate Ag typing of these transfused recipients by traditional serological methods is often challenging.

Red blood cell (RBC) transfusions have played an essential role in allowing sickle cell disease (SCD) patients to live longer. However, their use is complicated by the high incidence of RBC alloimmunization caused by serological compatibility testing, making identifying compatible RBC products complex and associated with delayed hemolytic transfusion reactions. These drawbacks in serological phenotyping have not been researched enough, especially in this environment. Thus, there is a need for a study of this nature to compare this serological compatibility method with molecular genotyping among patients with sickle cell disease in Ilorin. This study will help to demonstrate the relevance of molecular genotyping to allow the determination of the true blood group genotype and by assisting the identification of suspected alloantibodies in the patient, thereby preventing additional alloimmunization and serological typing discrepancies in the Ilorin metropolis.

The aim of this study is to compare molecular genotyping to serological compatibility testing methods among sickle cell disease patients attending a tertiary hospital in Ilorin. The objectives are to determine the sociodemographic and clinic-laboratory characteristics of Sickle cell disease recipients and their donors in Ilorin, and to compare serological compatibility testing methods with molecular genotype for any discrepancy.

MATERIALS AND METHODS

Study sites: The recipients' Samples were collected at the Sickle cell clinic, while those of the donors were done at the Blood bank, both at the General Hospital Ilorin, Kwara State (now Kwara State University Teaching Hospital (KWASUTH)). The Laboratory procedures were performed at the Inqaba Biotech PPP Molecular Laboratory, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun state.

Study Design: This study was a cross-sectional comparative design conducted on different

donor blood and sickle cell recipients who met the inclusion criteria at the general hospital Ilorin within a two-month period.

Selection Criteria: Donors whose blood was free from Transfusion-Transmissible Infections (TTIs) and Sickle cell disease recipients who were confirmed for blood transfusion and gave consent were recruited.

Sample Size Determination: This was calculated using the Cochrane formula

$n = z^2 p (1-p) / d^2$

where: n = sample size, z = the standard deviation at a 95% confidence interval (1.96), P = proportion of the population with the desired factor (adopted from the prevalence of SCD in Ilorin = 6.2% = 0.062 as reported by 23, q = 1-p (0.826), while d is the maximum allowable error (5%). Thus, 100 estimated participants were recruited for this study, comprising 50 SCD recipients and 50 donors.

Data Collection Tools: Data was collected using well-structured, self-administered questionnaires specifically designed to obtain information that was used to either include or exclude individuals from this study. A questionnaire was given to the participants or their caregivers through an interviewer-assisted administration method, which served as the major source of primary data for this study. To obtain the required number of study participants and objective data, a paper questionnaire will be made available to interested participants with a high level of participant safety and anonymity. The participants were informed of the study's goals and objectives. The consent forms were submitted with the questionnaire, and informed consent from the participant was sought. The gathered data were kept on a safe, password-protected I-drive for future reference.

Sample Collection and Processing: Blood samples (5mls) each from donors and SCD patients were taken into an Ethelene diamine tetra acetic acid bottle (EDTA). A 5ml sample from the SCD Patient was also taken into a plain bottle. It was transported to the laboratory immediately. The sample in the EDTA container was inverted several times and stored at 2-8oC. The samples in the plain bottle were separated, and the serum was kept for the serological procedure.

Serological Compatibility Testing:

Serological phenotyping was performed by routine erythrocyte blood group antigen typing by standard tube agglutination methods using polyclonal as well as monoclonal antibodies24The test tube method, which was considered to be more sensitive and reliable, was used for blood typing and compatibility procedures. Serologically, both forward and reverse grouping, antibody screening, and cross-matching procedures were carried out.

ABO and Rh typing: ABO and Rh typing were performed by a validated technique with appropriate controls. Monoclonal anti-A and anti-B reagents were used. A1 and B cells were used for reverse grouping, and group O cells or an auto-control were included to ensure that reactions with A and B cells were not due to the presence of cold autoantibodies.

Antibody screening: Antibody screening was also carried out using an indirect antiglobulin test as the primary method. The patient's serum was tested against at least two individual screening cells, used individually and not pooled (10). The screening cells were blood of group O, encompassing the state's common antigens.

Crossmatching: Serological cross-matches were performed by standard manual tube hemagglutination methods using both donor blood and the patient's serum based on their ABO and Rh systems. The presence of natural and Immune antibodies that can result in incompatibility in the patient was investigated.

Molecular Genotyping

Sample processing: The EDTA anticoagulated blood sample were preserved at 40C in the refrigerator for about two months before processing.

DNA Extraction: Genomic DNA was extracted from whole peripheral blood anticoagulated with EDTA using a Quick-DNATM Miniprep extraction kit according to the manufacturer instructions. The reagents used include Genomic Lysis Buffer 50mls plus (250µl of Beta- mercaptoethanol, DNA pre-wash Buffer, DNA Wash Buffer or genomic binding Buffer, DNA Elution Buffer, Zymo-spin TM llCR Column, and Collection Tubes

DNA Purity check: The eluted DNA was subjected to a purity check to assess the concentration of the eluted DNA. The quality of the eluted DNA was checked using (A thermo-scientific Nanodrop spectrophotometer) for the concentration, purity at (A260/280), and impurity at (A260/230). The elute with a very high DNA concentration and high purity were run for polymerase Chain Reaction.

PCR Strategy: The multiplex PCR contains four primers. Two forward and two reverse primers, previously described by (Simsek et al.,) Rh607 for a sequence located in exon 4 (5′ ACGATACCCAGTTTGTCT 3′, sense primer, position 607±624) and Rh768 for a sequence located in exon 5 (5′TGACCCTGA GATGGCTGT 3′, antisense primer, position 768±751) was synthesized for RHCE antigen while Rh1228 for a sequence located in exon 10 of both RH genes (5′ TTTCCTCATTTGGCTGTTG 3′, sense primer, position 1228±1246); and Rh1478 for a sequence located in the three non-coding region of the RHD gene (5′ CATGGCTGTATTTTATTGTTGTAT 3′, antisense primer, position 1478±1455) was synthesized for RHD antigen.

Designed Primers for the study

Toll-Like Receptors	Forward Primer	Reverse Primer
Rhesus antigen CE (Exon 4 and 5)	ACGATACCCAGTTTGTCT	TGACCCTGA GATGGCTGT
Rhesus antigen D (Exon 10)	TTTCCTCATTTGGCTGTTG	CATGGCTGTATTTTATTGTTGTAT

PCR Amplification: The PCR was performed in a master cycler (Nexus GS X 1) with approximately 5µl of genomic DNA in a final volume of 20µl containing 1.0µl each of the four primers, 12.5µl of the Master mix, and 3.5µl of double-distilled water. PCR conditions were 94^0C for 5 min, followed by 29 cycles of 94^0C for 1 min, 58^0C for 1 min, 72^0C for 1.5 min, and a final extension at 72^0C for 7 min.

Gel Electrophoresis: The PCR products were separated by electrophoresis on a 2% agarose gel containing 3µl of ethidium bromide and were visualized under ultraviolet light and the gel images were documented for each sample (25).

The reagents used include electrophoresis buffer (prepared by adding 100 ml of Tris borate EDTA plus 900 ml of double-distilled water), Agarose gel, and Ethidium bromide.

Statistical Analysis: After collecting data, it was checked for integrity and consistency before being coded. Data collected was double

entered in the Statistical Package for Social Sciences (SPSS) version 27 using a well-defined codebook (26). Data were cleaned for errors, cross-checked, and corrected as applicable. Descriptive statistical methods were used to summarize data on sociodemographic characteristics. The result was expressed as a mean ± SD. Chi-squared tests were employed to assess the comparison between molecular and serological typing. In the Chi-squared test, variables with $p \leq 0.05$ were considered significantly different (27).

Ethical Consideration: Ethical clearance was obtained from the Ethical Review Committee of Kwara State Ministry of Health, Nigeria, with the ethical approval number **ERC/ MOH/2024/02/189.** Informed consent was also obtained from all participants following the standards of human experimentation. This research will adhere to ethical guidelines for academic integrity and participant protection. Firstly, all secondary data used were adequately cited to avoid plagiarism. Before data collection, participants were fully informed of the study's purpose and procedures and required to provide consent. They were also notified of their right to withdraw consent at any time.

RESULTS

The mean age of the recipients is 7.6 (±4.3) years. Most (40%) have completed nursery school and are currently in primary school. The Mean Body weight is 23.6 (± 8.2) Kg. Figure 1 shows the number of previous blood donations. 16 (32%) have donated blood within 4-6 months, while 14 (28%) are just donating for the first time. Figure 2 shows the number of previous blood transfusions. About 30 (60%) have been transfused at least twice, regarding the history of blood transfusion reaction episodes. About 8 of the 50 (16%) have had a blood transfusion reaction before, though mostly mild. .

Table 1 shows the blood groups of the recipients and the donors, along with the recipients' Indirect Antiglobulin Testing (IAT) and the recipient-donor serological compatibility results. The majority of the recipients (52%) are of O Rh D positive blood group, as are the donors (56%). All the recipients had negative IAT, and all the recipient-donor serological matching was compatible.

Table 2 describes the Weiner's Rh antigens and phenotypes of the recipients and the donors, as well as the Fisher-race phenotype of the recipients and donors. 29 (58%) recipients are Rz Antigen with 33 (66%) of R_1R_2 phenotype. However, 21 (42%) are Rz antigens of the donor, with 20 (40%) of the rr phenotype. Similarly, in the fishers race of the recipients, 26 (52%) are DCe/DcE, compared to 20 (40%) dce /dce of the donor.

Figure 3 explains the molecular compatibility tests. The majority, 40 (80%) of the recipient-donor compatibility, are RHCDE /R1R2 compatible, and relatively 5 (10%) were not compatible molecularly. Also, only 22 (44%) were compatible with both RhCE /r'ry and Rh D/R0R0, while 3 (6%) were incompatible with both the recipients and donors. Comparison of Serological and Molecular Compatibility

When the serological and molecular results for RhD, RhC/c, RhE/e, and compatibility testing for *SCD* patients were correlated, there were (n=10) discrepancies between serological phenotyping and molecular *RHCDE* genotyping. The result shows that among the ten discrepancies, four were due to Rh D antigens, four were also due to RhCE antigens, and two were due to both RhCDE antigens. However, there was complete compatibility between serological and molecular compatibility of fourty sickle cell disease patients.

Figure 3 compares the serological and molecular compatibility results; it was found that out of the 50% of the samples that were compatible serologically, only 40% were compatible with the molecular procedure.

The remaining 10% of the samples were not compatible at all with RhCDE/R1R2 antigens and were not safe for transfusion into sickle cell disease patients.

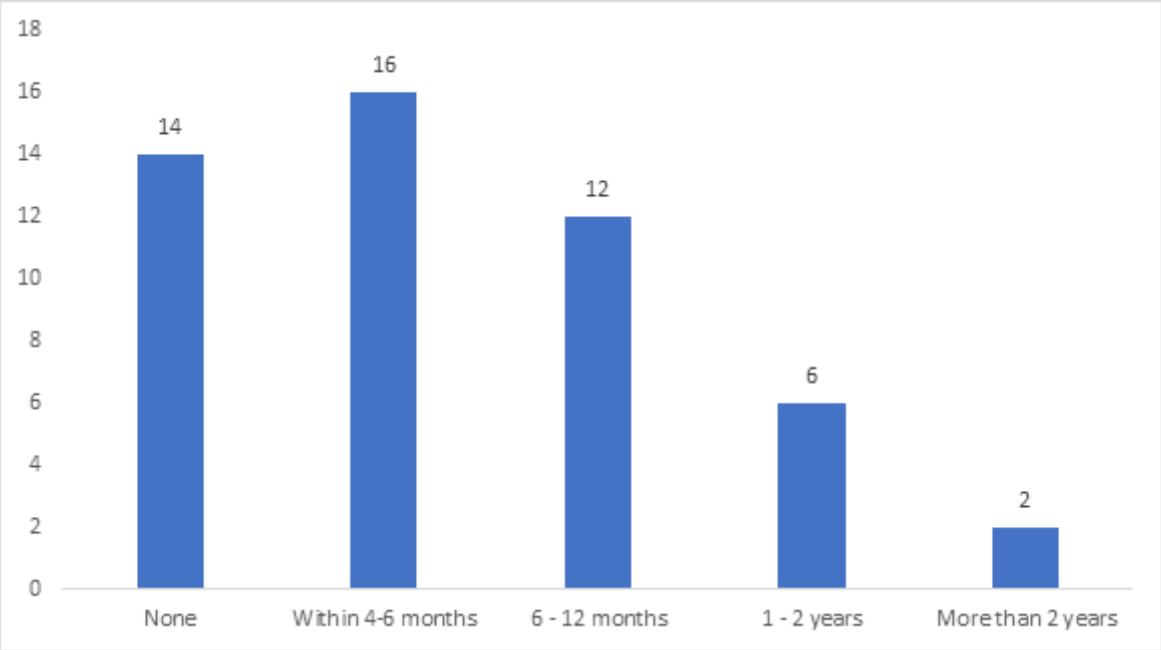

Figure 1: Number of Previous Blood Donation

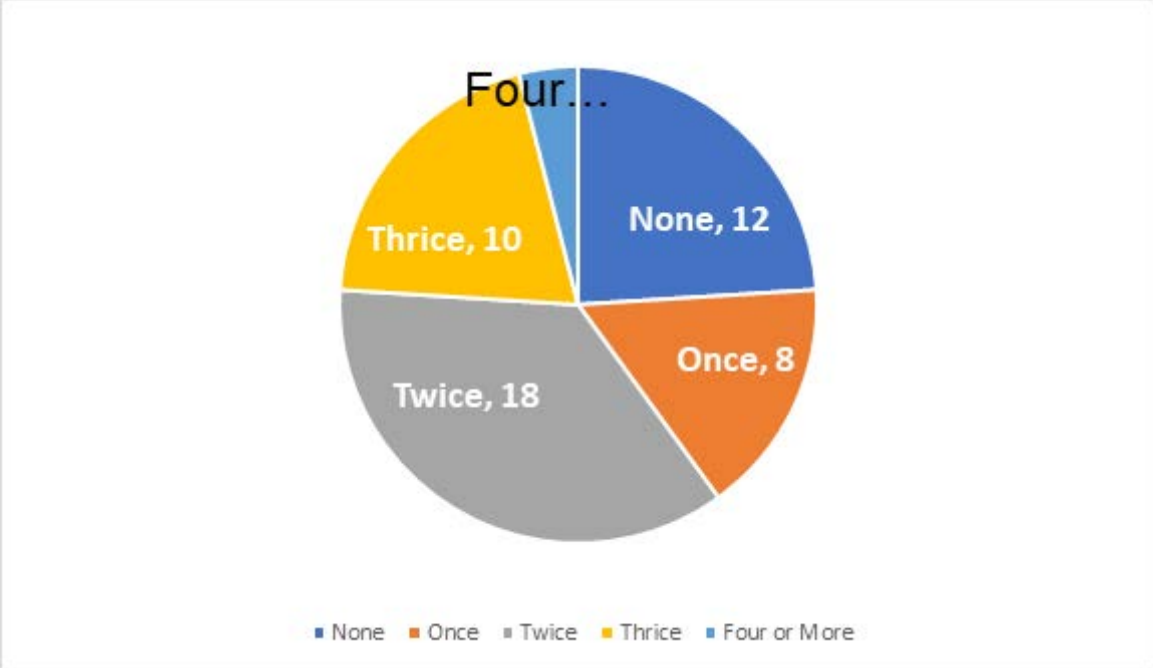

Figure 2: Number of previous blood transfusions

Table 1: Serological Characteristics of the Recipients and Donors

Characteristics		Recipient N (%)	Donor N (%)
Genotype			
	SS	**50 (100)**	**0 (0)**
	AA	**0 (0)**	**50 (100)**
Blood group			
	A Rh D Positive	2 (4)	2 (4)
	B Rh D Positive	12 (24)	12 (24)
	B Rh D Negative	2 (4)	0 (0)
	AB Rh D Positive	6 (12)	4 (8)
	O Rh D Positive	26 (52)	28 (56)
	O Rh D Negative	2 (4)	4 (8)
Indirect Antiglobulin Test			
	Negative	50 (100)	
	Positive	0 (0)	
Serological Compatibility			
	Incompatible		0
	Compatible		50 (100)

Table 2: Molecular Characteristics of the Recipients and Donors

		Recipient N (%)	Donor N (%)
Weiners Rh Antigen			
	R0	15 (30)	17 (34)
	Rz	29 (58)	21 (42)
	R	5 (10)	10 (20)
	r′r′′	1 (2)	2 (4)
Weiners Phenotype			
	R0R0	11 (22)	10 (20)
	R1R2	33 (66)	16 (32)
	Rr	5 (10)	20 (40)
	r′r′′	1 (2)	4 (8)
Fishers-race phenotype			
	Dce/Dce	18 (36)	10 (20)
	DCe/DcE	26 (52)	16 (32)
	dce/dce	5 (10)	20 (40)
	dCe/dcE	1 (2)	4 (8)

Figure *3:* Comparison of Serological Test with Molecular Compatibility Tests

DISCUSSION

The study's findings are discussed following the study objectives, which include the socio-demographic and clinical characteristics of the participants, the serological compatibility reports, the molecular compatibility outcomes, and their comparison.

The mean age of the recipient sickle cell disease patients is 7.6 (± 4.3) years, while many are currently in Primary school. The Mean Body weight is 23.6 (± 8.2) Kg. This mean age reflects the distribution of Sickle cell disease patients who receive blood transfusions in this hospital, as it is usually in childhood ages that they have crises and other events that indicate the need for transfusion. Also, almost two-thirds of them have been previously transfused. Sickle cell disease is a genetic disorder affecting haemoglobin production, leading to re-occurring episodes of anemia, pain, and increased risk of infections. Hence, a key reason why a sickle cell disease patient receives blood around this time is in the Management of Sickle cell anaemia because Sickle cell disease can lead to chronic anaemia due to the rapid sequestration and breakdown of sickle-shaped red blood cells (23). Transfusions help increase the number of healthy red blood cells, improving oxygen delivery to tissues (28). Another common reason is in the management of acute pain crises. Transfusions can alleviate pain during pain crises by improving oxygenation and reducing sickling episodes (29). Furthermore, some patients may develop other health issues, such as severe splenic dysfunction, making transfusions necessary for managing these conditions. Overall, blood transfusions are a crucial part of the treatment plan to manage symptoms and improve the quality of life for individuals with sickle cell disease.

About 1 in 6 of the recipients have had a blood transfusion reaction before, though mostly mild. As much as blood transfusion is a crucial aspect of SCD management, reducing complications and improving quality of life, transfusion reactions can occur due to major compatibility issues (30). Mild transfusion reactions can be caused by several specific factors, such as haemolytic reactions, which can occur due to the recipient's immune response to white blood cells or cytokines in the transfused blood, resulting in fever and chills (31). Also, some recipients may have mild allergic reactions to components in the donor blood, leading to symptoms like itching or rash. Similarly, repeated transfusions may lead to iron overload in the body, causing reactions like transfusion reactions. Moreover, Pre-existing antibodies in the recipient's blood may react against antigens in the transfused blood, resulting in mild symptoms (32). Lastly, minor antigen mismatches (such as non-ABO/RhD blood group antigens) can trigger mild reactions even with serological compatibility. Hence, understanding these factors can help healthcare providers manage and mitigate risks associated with blood transfusions.

This study found a complete compatibility between serological and molecular compatibility of sickle cell disease. However, we discovered discrepancies in ten out of the pairs, yielding a sensitivity of 80%. Four of these were due to Rh D antigens, another four resulted from RhCE antigens, and the last two were due to RhCDE antigens. The discrepancies between serological and molecular compatibility can arise from several factors (33). First, the serological tests primarily check for specific blood group antigens, which may not always reflect the underlying genetic makeup (34). Genetic mutations or variations in the antigen expression can lead to discrepancies between what is detected serologically and the actual genotype. Also, serological testing typically focuses on significant blood group systems (like ABO and Rh). However, many minor blood group antigens might not be assessed. If a recipient has antibodies against these minor antigens, a transfusion may still result in reactions despite serological compatibility (35). Furthermore, some antigens may be

expressed in red blood cells at low levels. Suppose these antigens are not detected serologically (due to their subtle expression). In that case, molecular testing might reveal the presence of these antigens, leading to an apparent incompatibility (36). Lastly, if molecular testing assesses a broader range of antigens compared to serological testing, it may identify potential incompatibilities that serological methods overlook (37,38). In summary, while serological and molecular compatibility assessments are critical for safe transfusions, their inherent differences and limitations can sometimes lead to disparities in compatibility results. Understanding these factors is essential for healthcare providers to manage possible risks effectively. This calls for the necessity of molecular testing for the samples before blood transfusion to prevent the transfusion reactions that may occur.

CONCLUSION

This comparative study between molecular and serological compatibility testing among sickle cell disease patients in Ilorin has provided valuable insight into the effectiveness and limitations of both methods. The results demonstrate that serological compatibility testing methods have limitations in detecting some clinically important antigens that can lead to transfusion reactions. In contrast, Molecular Compatibility testing methods have higher accuracy and sensitivity in detecting all forms of clinically essential antigens. Thus, Molecular compatibility testing offers superior sensitivity, specificity, and accuracy compared to serological compatibility testing methods. These findings have significant implications for improving transfusion safety and patient outcomes.

Recommendations: We recommend the following:. First, serological compatibility procedures should be confirmed by molecular methods. Also, laboratories should adopt molecular compatibility testing as the gold standard for sickle cell disease patients requiring blood transfusion. Furthermore, stakeholders should ensure molecular testing is cost-effective for sickle cell disease patients, especially those in need of multiple transfusions. Finally, this study is also recommended for replication with modification at other regions of the Nation, for it has a lot to contribute to knowledge and advancement of the rapidly developing field of Haematology and Blood Transfusion Serology.

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