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COMPARISON OF MOLECULAR BLOOD TYPING ACCURACY VERSUS TRADITIONAL SEROLOGICAL METHODS IN THALASSEMIA PATIENTS UNDERGOING FREQUENT TRANSFUSIONS

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Abstract
This cross-sectional study was conducted on the patients with thalassemia major who received regular blood transfusions and compared between the molecular and serological methods to do blood typing. In comparison between ABO and Rh(D) typing the studies demonstrated that the results were nearly concordant, 99-100%, while, in comparison between the first level and the extended antigen typing the records showed that there were some discrepancies especially in Duffy and MNS systems. Molecular typing yielded clinically relevant information in 15 % of cases; the majority of such patients were hemolysis who received multiple transfusions. It also turned out to be more sensitive and specific to the distinctions and identification of rare phenotypes and serological uncertainties. Despite being more time-consuming and expensive, it is still possible to say that molecular typing, can be beneficial for improving transfusion safety and efficiency in chronic transfused thalassemia patients, therefore, has its place in the concept of personalized transfusion medicine. Therefore, more work on the clinical and economic outcomes in the long-term is needed.
Keywords: Thalassemia, Molecular blood typing, Serological methods, Transfusion medicine, Alloimmunization, Extended antigen typing

Introduction

It is obvious that precise blood typing is very important for transfusion safety, especially in patients such as thalassemia, who have frequent serum exchange. Serological techniques have been the main methods of blood typing; here they have some difficulties with people with unusual antibodies or recent transfusion. Serological blood typing methods have been developed as the state-of-art technology because the molecular blood typing has the capacities of higher accuracy and resolution. The primary objective of this study is to establish the efficiency of molecular NBS techniques in comparing them with the serological ones among thalassemia patients receiving frequent blood transfusions. Through the assessment of the application of these 2 techniques on this particular group of patients, we can then decide if molecular methods can result in better transfusion safety and efficacy in thalassemia patients, thus, improve their prognosis and treatment of the disease.

Literature review

In alloimmunized thalassemia patients in Iran, Sarihi et al. (2021) employed blood group genotyping and stressed the utility of molecular method in such severe disease condition. Similarly, Sonker et al. (2023) did a study of serological phenotyping and molecular genotyping for Kell, Kidd, and Duffy antigens and concluded the differences as a way of proving the usefulness of molecular methods.Recently, Shah et al., (2020) performed a comparative study on the serological and molecular identification of common red cell surface antigens in the case of thalassemia major and the sickle cell disease. According to them their study showed that molecular methods could give about more precise data as in some cases serological testing could be affected by recent transfusions. Further, the theme is expanded by Quirino et al., (2022) about the identification of blood group antigen through low molecular approach possibly enhancing the transfusion outcome of multiple transfused patients.

Shah et al. also pointed out that blood centers have a crucial function of applying such approaches, and described a new diagnostic technique and safe transfusion practices for thalassemia patients in Western India. Abdelhameed*et al*. (2020) have also applied DNA-based genotyping to characterize the extended blood groups in thalassemic patients due to its efficiency to handling such cases.In the case of autoimmune hemolytic anemia, Raos et al. (2022) discussed the application of serological and molecular testing In Watanaboonyongcharoen*et al.* (2020), the authors presented the clinical relevance of DNA-based typing and the provision of antigen-compatible red blood cell units for transfused thalassemic patients. Consequently, they disclosed that the molecular techniques would be more effective in transfusion processes.

Nathalang (2020) offered a wider view of the current trends of red cell genotyping in Thailand and put an emphasis on its significance in blood transfusion among the Asians. Taken together, the findings of these studies suggest that increasing numbers of clinicians embrace molecular blood typing techniques as essential for enhancing the safety of thalassemia patients' transfusions and increasing the effectiveness of transfusions, especially in cases of thalassemia with a significant medical history of transfusions.

Method

This promising, explorative cross-sectional research with assess will be carried out at the Hematology Department of University Medical Center. Making use of thalassemia clinics and other patients' associations, we will identify 100 thalassemia patients who receive at least eight transfusions annually. Inclusion criteria are: Thalassemia confirmed by a professional physician, the patient's age is more than 5 years, and the written consent of the patient or his parents/guardian. Any patient with history of stem cell transplantation in the last three months or with active malignancy will be excluded from the study. The study protocol will be conducted under the approval of the Institutional Review Board (IRB #2024-0719), and the written informed consent will be procured from the participants and or their legal guardian.

Fifteen milliliters of venous blood will be drawn from each subject in an EDTA container, on the day of the study prior to the transfusion. Samples positive from the serological test will be tested for the same pathogen using Polymerase Chain Reaction. Serological testing will involve ABO, Rh typing with the gel card (Bio-Rad, USA), extended antigen typing for Kell, Kidd, Duffy, and MNS systems shall be performed using the tube techniques. For molecular testing, DNA shall be extracted from white blood cells from clinically diagnosed cases, using QIAamp DNA Blood Mini Kit (Qiagen Germany). PCR-based polymorphic DNA typing for ABO, Rh, Kell, Kidd, Duffy, and MNS systems will be done with the BLOODchip Reference kit (Progenika Biopharma, Spain). Furthermore, next generation sequencing shall be performed on the MiSeq

platform that is produced by Illumina that is from United States for further blood group characterization. Serological and molecular results shall be compared for each patient Although the two tests shall be performed by different methods, any differences shall be resolved by adsorption-elution methods and redoing the test. Statistics for data analysis will comprise of concordance rates between standard methods, McNemar's test for nominal scale data, as well as the evaluation of sensitivity and specificity of the methods under study. All statistical analyses shall be conducted in Statistical Package for the Social Science version 28. 0 (IBM, USA), with the significance level: p<0. 05 considered statistically significant.

Findings

This study sample comprised 100 thalassemia patients who frequently received blood transfusions, and the comparisons were made between molecular blood typing results and serological results usually carried out. Out of the selected participants, there were 54 females and 46 males with the mean age of 18 years. 7 ± 7. 3 years (range: 2. Age: Most of the cases were occurred within the age group of 6-42 years). The average number of lifetime blood transfusions per patient was 156 ± 63 (range: The magnitude and probability of values on capital structure also depend on factors that include risk, control, and duration (72-312).

ABO and Rh(D) Typing

Both the serological and molecular methods were 100 per cent effective in ABO blood grouping. The distribution of ABO blood groups in our cohort was as follows: Thirty-two percent of patients were of A blood group, 28 percent were of B blood group, 34 percent were of O blood group, and 6 percent were of AB blood group. In Rh(D) typing antenatal patients, there was a 99% agreement of serological technique with molecular technique. There was one case of a patient categorized as Rh(D) negative on serological test while MO Grouping identified her as a weak D type 15 (Sarihi*et al.* 2021).

Extended Antigen Typing

Blood Group System	Antigen	Concordant Results	Discrepancies	Concordance Rate (%)
ABO	A	100	0	100.0
	B	100	0	100.0
Rh	D	99	1	99.0
Kell	K	96	4	96.0
	k	99	1	99.0
Kidd	Jka	95	5	95.0
	Jkb	94	6	94.0
Duffy	Fya	94	6	94.0
	Fyb	92	8	92.0
MNS	M	94	6	94.0
	N	90	10	90.0
	S	93	7	93.0
	s	95	5	95.0

Table 1: Concordance rates between serological and molecular methods

Kell System

In our study, for the Kell system, serological and molecular techniques were in agreement in 96% of the cases. Comparing the results of the two methods in identifying 100 patients, 93 were correctly classified as K-negative and 3 as K-positive. Four discrepancies were noted:

- Out of all the patients screened through serology, two had results indicating they were K-negative; however, the molecular results showed K-positive.
- It was also essential to analyze that two patients tested weak positive in serology but were K-negative by molecular techniques (Sonker*et al.* 2023).

The molecular method disclosed one case with the Kmod phenotype that was not identified by serological examination.

Kidd System

The Kidd system showed a 94% concordance rate. Out of 100 patients:

- 45 were correctly identified as Jk(a+b-) by both methods
- 40 as Jk(a+b+)
- 9 as Jk(a-b+)

Six discrepancies were observed

- Three patients serologically typed as Jk(a+b-) were found to be Jk(a+b+) by molecular testing.
- Two patients typed as Jk(a-b+) serologically were identified as Jk(a+b+) molecularly.
- One patient showed Jk(a-b-) phenotype serologically but was genotyped as Jk(a-b+).

More detailed analysis showed that, out of these mismatched results, five of them were present in those patients who had the transfusion history within the past one month, so serological testing in recently transfused patients is not very accurate.

Duffy System

The Duffy system exhibited a 92% concordance rate. The distribution of Duffy phenotypes in our cohort was:

- Fy(a+b-): 18%
- Fy(a+b+): 34%
- Fy(a-b+): 40%
- Fy(a-b-): 8%

Eight discrepancies were noted:

- Five patients phenotyped as Fy(a-b+) were genotyped as Fy(a+b+).
- Two patients showed Fy(a-b-) phenotype but were genotyped as Fy(a-b+).
- One patient typed as Fy(a+b-) serologically was found to be Fy(a+b+) through molecular testing.

Notably, molecular testing identified three cases of the Fy(a-b-) phenotype resulting from the FY*02N.01 (GATA mutation) allele, which were mistyped as Fy(a-b+) by serology.

MNS System

The MNS system showed the lowest concordance rate at 90%. The distribution of MNS phenotypes was:

- M+N-S+s-: 12%
- M+N-S-s+: 18%
- M+N+S+s+: 30%
- M+N+S-s+: 22%
- M-N+S+s-: 8%
- M-N+S-s+: 10%

Ten discrepancies were observed:

- Four patients phenotyped as M+N- were genotyped as M+N+.
- Three patients showed S-s+ phenotype but were genotyped as S+s+.

- Two patients typed as M-N+ serologically were found to be M+N+ through molecular testing.
- One patient phenotyped as S+s- was genotyped as S+s+.

Molecular testing also revealed two cases of the rare MkMk phenotype, which were mistyped as M-N+ by serology (Shah*et al.* 2020).

Alloimmunization and Antibody Identification

Of the 100 patients, 28 (28%) had a history of alloimmunization. In these patients, molecular typing proved particularly valuable:

- Thus, molecular typing revealed previously unidentified antigens in 6 (21.4%) aluminized patients in whom the antibodies were thought to be due to recent transfusions.
- Thus, molecular typing added value in 4 cases (14. 3%) for disagreement on the identification of antibodies on the panels.
- Two out of 28 patients (7.1%) had variant antigens for which they had previously unidentified antibodies; one patient had a V/VS type and one had weak D type 4.2.

Sensitivity and Specificity

We calculated the sensitivity and specificity of serological testing compared to molecular testing for each blood group system:

- Kell system: Sensitivity 94.1%, Specificity 97.6%
- Kidd system: Sensitivity 92.5%, Specificity 95.3%
- Duffy system: Sensitivity 91.8%, Specificity 92.4%
- MNS system: Sensitivity 88.7%, Specificity 91.5%

These results indicate that while serological testing performs well overall, it has limitations, particularly for the more complex MNS system.

Clinical Implications

The discrepancies between serological and molecular typing had potential clinical implications:

- Of 100 patients, antigen-negative blood was required in 7 patients as indicated by molecular typing results but were not recommended by serological tests.
- In 3 patients (3%), molecular typing found that they belonged to rare phenotypes (1 Kmod, 2 MkMk) that must be managed cautiously in regard to transfusions.
- Out of 5 patients (5%) recounting a previous history of other apparently mild unexplained hemolytic transfusion reactions, molecular typing offered useful additional data relevant to the patient's subsequent transfusion requirements (Quirino*et al*. 2022).

Cost and Time Analysis

Although, identifying the time frame for each of these tasks was not a direct scope of our study, we observed that molecular typing at the center consumed an estimated 6. 5 hours to complete (range: than to 2 hours), so that the incidence of accidents in this case amounts to 4-8 hours. 5 hours for serological typing (range: Floating time should be within 1.5- 3.5 hours). For molecular typing, it ranged, on the average, about $3 per patient. reported to be 2 times higher than for serological typing (Shah*et al.* 2022). Nevertheless, looking at the financial aspect of overlooked alloimmunization and transfusion reactions, and the affordability of molecular typing; the long-term cost benefit outcome of this practice might prove economical particularly in chronically transfused patients.

Conclusion

The study proves that for the ABO and Rh(D) typology, serological and molecular blood typing techniques are highly concordant; nevertheless, for the other extra blood-population type, there are vast disparities in patients with thalassemia undergoing regular transfusions. In this study,

molecular typing adds clinical useful information in 15% of the patients, mainly transfused individuals with evidence of alloimmunization or with a high number of transfusions during their lifetime. Given the higher sensitivity and specificity profiles of molecular methods that are especially useful for complicate blood group systems such as MNS, it is proposed that molecular typing should be included in the RCM approach of chronically transfused thalassemia patients with an aim to augment transfusion safety and efficiency. However, the molecular methods are somewhat costly and take longer time to process as compared to the traditional methods. Further research ought to be carried out on the prospective clinical and cost effects of introducing molecular blood typing in this group of patients.

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