Distribution OF ABO Predicated Phenotypes among Voluntary Blood Donors applying Next Generation Sequencing - Insights from Kenya

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Abstract

Background: ABO system is a major determinant for blood transfusion and organ transplantation incompatibility. ABO histo-blood group antigens are sugars attached to glycolipids and glycoproteins on the surface of human cells and readily recognizable by antibodies which are naturally present in each person plasma/serum. Laboratory testing for ABO antigens and isoagglutinins is essential for safe and effective transfusion and transplantation. Testing for ABO antigens has traditionally depended on serologic testing which is limited in the determination of the weak subtypes risking the recipients to alloimmunisation especially those we are receiving ongoing transfusions with red cell such as sickle cell disease and oncology. . It has also impacted negatively in the management of rare red cell units. However, there is increasing need for evaluation of genetic analysis of ABO antigens, to enable evaluation of ABO blood group in cases where serologic testing may be ambiguous or impossible to accurately determine the presence of these antigens; plus having such data in a country. Thus, there is need to investigate the genotypes/Alleles responsible for ABO blood group phenotypes in the blood donor population to enhance safety in transfusion and transplantation practices.

Methods: The study site was Kenya National Blood Transfusion Service and Red Cross lifeblood Brisbane, experimental design was employed, sample size determination was determined using Slovin's Formula Sampling Techniques, and purposeful sampling method was employed to achieve a representative sample. Next generation sequencing was employed to determine red cell alleles and predicted phenotypes. Sequencing was performed using the Illumina MiSeq platform with 12-plex pools and standard 300-cycle V2 chemistry.

Results: Out of the total 119 samples sequenced, ten phenotypes combinations were predicted: O (52.3%), A1 (16.2%), B (10.5%), A1 or B (6.8%), A1 or A2 (5.8%), A1 or Ax/Aweak (1.6%), B or B3 (1.6%), A1B (2%) A2B (2%).and Bweak (0.5%). This can be expressed as (O> A1> B > A1 or B > A1 or A2 > A1 or Ax/Aweak > B or B3 > A1B > A2B > Bweak.

Conclusion: The study findings elucidated the genotypes that are responsible for the ABO thirteen phenotypes in the identified Kenyan population. This data forms the basis or evidence to advocate for a blood group reference laboratory and a red cell panel that is African specific.

Keywords: ABO, Genotyping, Phenotypes, Antigens, Next generation sequencing

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1. Introduction

ABO system is a major determinants for blood transfusion and organ transplantation incompatibility. ABO histo-blood group antigens are sugars attached to glycolipids and glycoproteins on the surface of human cells and readily recognizable by antibodies which are naturally present in each person plasma/serum. ABO histo-blood group incompatibility can cause hyper acute rejection of solid organ allografts via isoagglutinins binding endothelial cell blood group antigens and initiating thrombosis. ABO histo-blood group compatibility is also a primary immunologic consideration for donor-recipient histocompatibility (Usmani et al., 2024). Accurate matching of ABO antigens of donor and recipient is critical in transfusion and transplantation therapy. For the identification of the ABO phenotypes, serological methods are routinely applied. However where there is no extended phenotyping, the weak subtypes expressions are usually missed be it in donor or recipient and this results in alloimmunisation incase of absence of these weak types in the recipient. To supplement serological typing, several medium-to high-throughput molecular typing methods have been developed to enhance accurate detection. (Lang et al., 2016). Importantly, the ability to test for antigens for which there are no serologic reagents is a major medical advance to identify antibodies and find compatible donor units, and can be life-saving (Daniels, 2023).

Blood transfusion is one of the most commonly administered therapies in clinical medicine. Current pretransfusion testing includes matching the patient and donor for ABO and RhD using approaches that have served the profession well, but that have not materially changed for .60 years. It is an exciting time in transfusion medicine because the field is poised to benefit from a genomics approach not only for recipient and donor compatibility determination, but for donor recruitment, donor health, product storage characteristics, and characterization of the metabolomics components of transfusion.

Alloimmunization is one of the risk of blood transfusion in the absence of mitigation strategies. It is commonly recognized that some level of antigen matching of the patient and donor beyond ABO would improve outcomes and reduce alloimmunization among patients with SCD or other anaemia. Therefore, it is imperative to expand the technique in the era of precision medicine by applying next-generation sequencing to leverage the knowledge accessible through genomes.

This method can also be applied in blood group genotyping can also be used to detect blood group chimerism and minor subtypes (Westhoff, 2019 ., Kim et al., 2021).

Genotyping can be applied to determine blood groups phenotypes beyond ABO transfused patients, where it is not possible to do the testing serologically because of the presence of transfused red cells especially in those receiving ongoing transfusions. While it is a standard practice that these patients are serologically investigated prior commencing the transfusion processes, this does not always happen. Knowledge of the patients' extended blood groups means that matched blood can be provided in an attempt to prevent them from forming immunoglobulin's (antibodies). Genomic testing can be applied to determine blood group antigens on red cells that have been coated with antibodies *in vivo* and provide a positive direct antiglobulin test (DAT), making serological testing difficult. This is particularly useful in helping to identify underlying alloantibodies in patients with autoimmune haemolytic anaemia (Daniels, 2023). Therefore, there is need to investigate the genotypes/Alleles responsible for ABO blood group phenotypes in the blood donor population to enhance safety in transfusion and transplantation practices.

2. Materials and methods

2.1 Study site

The research was conducted at the Kenya National Blood Transfusion Service (KNBTS), which serves as a pivotal institution in the country's healthcare system, responsible for ensuring an adequate and safe supply of blood for medical purposes. KNBTS operates through various regional centers and blood donation drives across Kenya, facilitating blood collection, testing, processing, and distribution.

2.2 Target population

The study focused on volunteer donors who contributed blood to KNBTS. Additionally, the research included donated blood samples that were leftover from routine blood collection procedures. These samples are valuable resources for scientific investigation, providing insights into various aspects of blood physiology, transfusion medicine, and disease detection.

2.3 Study design

An experimental design type was employed for this study. Experimental designs are characterized by the manipulation of independent variables to observe their effects on dependent variables. In the context of blood transfusion research, experimental designs allow researchers to investigate interventions, treatments, or procedural changes aimed at improving blood safety and efficacy.

2.4 Sample size determination

The study employed purposeful sampling method to achieve the sample size

n = N/(1 + Ne2)

Where:

n = Number of samples=120

N = Total population = 5%

e = Error tolerance (level)

n = 120(total samples)

N = 5% (0.005) (CI)

120/(1+120*0.05)=120/(1+121*0.05*0.05)

=120/1.3025=92.1305

Sample size =92

Samples collected in 4mls ETDA tubes, weak D positive and D negative, non-reactive for transfusion transmission infections (TTIs), with no haemolysis were selected for the study

2.5 Sampling procedure

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2.6 Data collection, assurance and control

In this study, the integrity and volume of samples (4mls of blood samples collected in ETDA were assessed for consistency and conformity; data quality management was adhered to during data collection, storage, processing, analysis and publication. The collection processes involved selection and acquisition of blood donor samples. Unique codes were generated for labeling the selected samples which were entered into an excel sheet ensuring the right codes were indicated correctly. These unique codes were used and maintained throughout the sample handling and processing (manual genomic DNA extraction), samples shipment, quantification, sequencing, annotating, analysis, result interpretation, publication and storage.

Molecular genotyping: Manual Genomic DNA extraction was accomplished by using QIAamp whole blood DNA mini kit (250 51106) as per manufacturer's instructions (Qaigen Germany). Library preparation was performed using a custom blood grouping enrichment panel (Roulis et al 2020), and the Illumina DNA prep Enrichment protocol as per manufacturer's instructions (Illumina Inc., San Diego, CA, USA). Next generation sequencing was performed using a targeted custom blood group sequencing panel on an Illumina MiSeq9. JK genotype and predicted phenotype were determined independently by two research scientists using variant calling format files and ISBT Blood Group Allele Tables to provide blood group genotype and predicted phenotype (Roulis, et al., 2022).

2.7 Data Management, analysis and presentation

Sequencing data Read mapping and variant prediction was performed on board the MiSeq using BWA-MEM (Li H. 2013) and GATK (McKenna, A et al., 2010), generating BAM and VCF files respectively. BAM and VCF files were submitted to the RBCeq webserver for analysis and reporting of blood group variants, alleles and phenotypes (Jadhao et al., 2022) and results displayed in table and figure forms.

2.8 Ethical Considerations

Study was conducted in accordance with the ethical guidelines and regulations of Mount Kenya University Ethical Review Committee, Ministry of Health Kenya, Australia Red Cross and National Commission for Science, Technology and Innovation (NACOSTI).

3. Results and Discussion

Out of the total 119 samples sequenced, ten phenotypes combinations were predicted (Figure 1& 2): O (52.3%), A1 (16.2%), B (10.5%), A1 or B (6.8%), A1 or A2 (5.8%), A1 or Ax/Aweak (1.6%),B or B3(1.6%), A1B(2%) A2B (2%).and Bweak (0.5%). This can be expressed as (O> A1> B > A1 or B > A1 or A2 > A1 or Ax/Aweak > B or B3 > A1B > A2B > Bweak

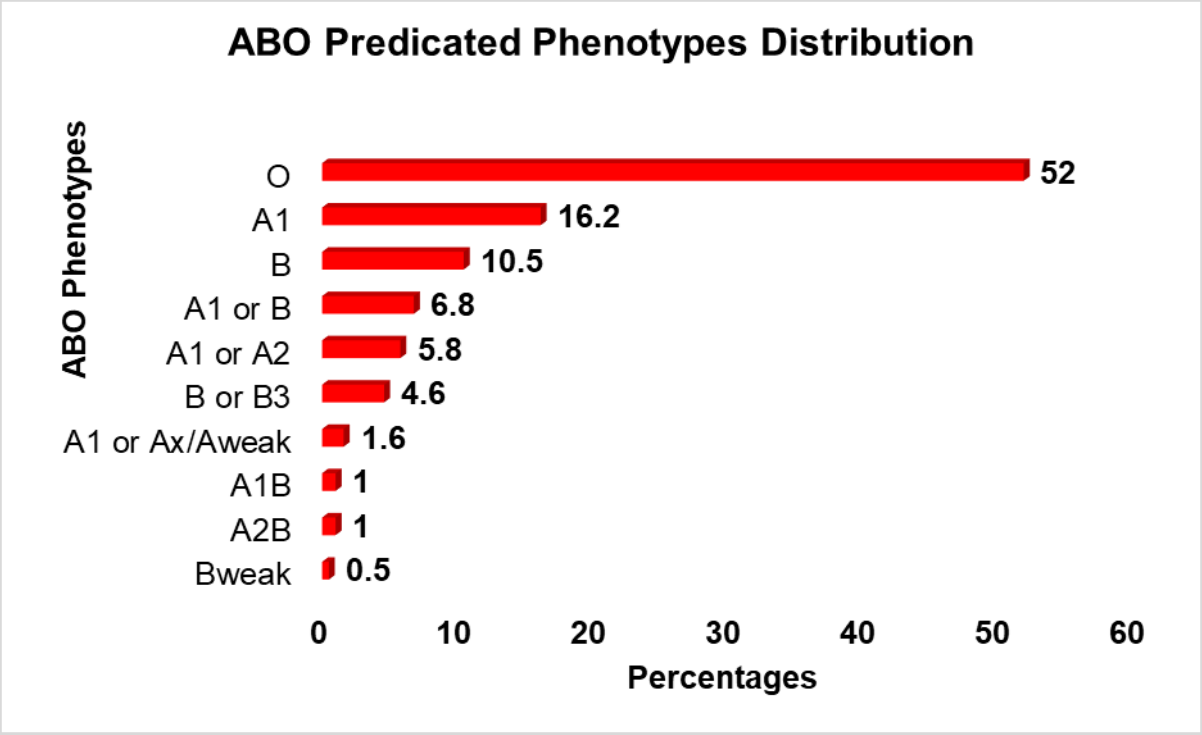

Figure 1: Showing ABO predicated Phenotypes percentages in the donor population of Kenya

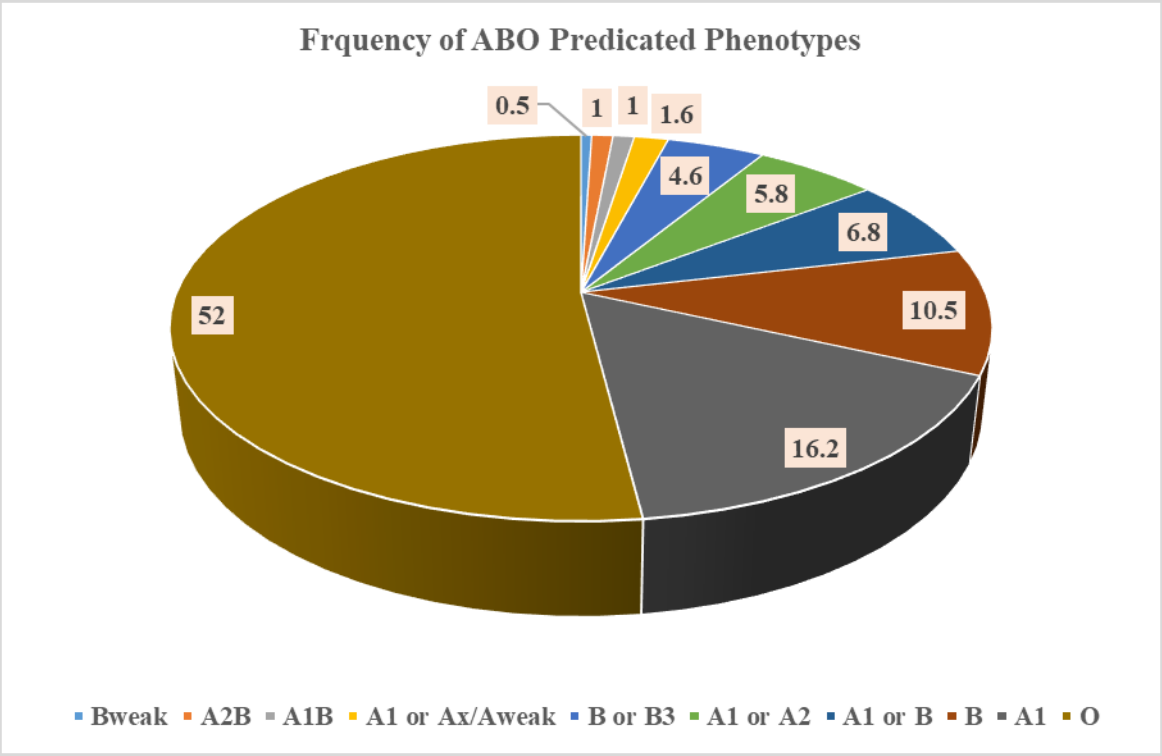

Figure 2: Showing ABO predicated Phenotypes Percentages in the donor population of Kenya

4. Discussion

The observed distribution of ABO predicated phenotypes in this cohort aligns with other studies in other populations. The high frequency of the ABO phenotype is consistent with the known prevalence in sub-Saharan Africa. The diversity of ABO genotype combinations reflects the complex nature of ABO allele variations and the importance of accurate genotyping in transfusion medicine. Moreover, the results highlight the significance of population-specific data when considering transfusion practices. Understanding the distribution of ABO alleles in different populations allows for tailored transfusion strategies and minimizes the risk of alloimmunization. Additionally, the genotypic and phenotypic information obtained from this study can contribute to the prediction of rare phenotypes and improve donor selection for individuals with specific ABO antigen requirements such as requiring ongoing transfusions (oncology and sickle cell disease). The findings also emphasize the importance of accurate genotyping in transfusion practice. Precise identification of ABO alleles and phenotypes ensures compatibility between donors and recipients, reducing the risk of adverse reactions and promoting transfusion safety. Incorporating advanced genotyping techniques, such as high-throughput next-generation sequencing, can enhance the efficiency and accuracy of ABO allele identification, particularly in cases where rare variants or novel alleles are present.

The molecular basis of ABO alleles provides valuable insights into antigen expression properties. Variations in allele sequences can lead to altered antigen phenotypes and affect transfusion compatibility. The identification of novel variants, as demonstrated in study, underscores the ongoing need for comprehensive characterization of ABO alleles. Continued research in area will contribute to expanding this understanding of the ABO blood group system and enable the development of improved strategies for blood matching and transfusion practice.

Conclusion

This study proved the utility of the NGS approach in detecting low levels ABO phenotypes (weak types).Our NGS assay accurately detected the minor subtypes of ABO phenotypes. Our study demonstrated that NGS-based blood group genotyping is an effective tool for identifying ABO subgroup alleles. Our findings thus suggest that NGS-based blood group genotyping can be applied to various immunohematology cases encountered in routine clinical practice

Recommendations

Based on the findings of study, the following recommendations are proposed: (i) Continued monitoring and analysis of ABO allele frequencies in diverse populations to ensure up-to-date and accurate transfusion practices. (ii) Implementation of advanced genotyping techniques, such as high-throughput next-generation sequencing, to improve the accuracy and efficiency of ABO allele identification. (iii) Collaboration between transfusion centers and research institutions to establish comprehensive databases of ABO allele variations and their associated phenotypes. (iv) Need to engage the training institutions to review existing training curriculums in line with current technological advancement.

(v) Capacity building of healthcare professionals involved in transfusion medicine to raise awareness of the importance of accurate genotyping and phenotype matching in blood transfusions and also to review the training curriculum to align with current technological advancement. (vi) More research is needed to determine the functional importance of ABO allele variants and how they affect transfusion compatibility and antigen expression.

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Conflict of Interest:
The authors declare no conflicts of interest.

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