EVALUATION OF KELL ANTIGEN PHENOTYPING VERSUS GENOTYPING IN EGYPTIAN MULTITRANSFUSED THALASSEMIC PATIENTS

Wafaa Ahmed El-Neanaey, Dalia Ahmed Nafea*,Reham Abdel Haleem Abo El Wafa, Salma Alaa El-Din Imbaby, Nermine Ahmed Moustafa El Sabbagh

Department of Clinical and Chemical Pathology, Department of Internal Medicine*, Faculty of Medicine, Alexandria University, Egypt

Introduction

Thalassemia is the most common hereditary single-gene disorder. Thalassemia patients typically require repeated RBC transfusions as their primary treatment option. The most common complication of RBC transfusion is alloimmunization against RBC antigens. The Kell blood group system is the third most significant immunogenic blood group system. Most studies have reported a high prevalence of antibodies against Kell system antigens in thalassemia patients. Accurate phenotyping of RBCs from transfused patients with thalassemia is a very complex process due to the presence of transfused donors RBCs in the recipient's circulation. Molecular assays are not influenced by the presence of immunoglobulins or transfused cells and can be used to determine RBC antigens even in recently transfused patients or those receiving multiple blood transfusions. Therefore, using molecular blood group genotyping can support transfusion decisions and prevent alloimmunization by an antigen-matched transfusion in these patients.

Aim of the work

The aim of this study is to estimate Kell antigen among Egyptian multitransfused thalassemic pateints and evaluate the usefulness of kell antigen genotyping in addition to conventional serological phenotyping in order to determine the pateints who will benefit from this technique.

Patients

The study was conducted on 60 multi-transfused patients with thalassemia major attending Alexandria University Children's Hospital and Hematology department of Alexandria Main University Hospital.

Methods

Patients were subjected to Full history taking,clinical examination, routine Laboratory investigations including (CBC, Blood group phenotyping), Determination of kell antigen by hemagglutination assays using PHENOKIT Anti-K (Kel 1) and Detection of Kell genotypes using PCR-restriction fragment length polymorphism (RFLP- PCR).1- Peripheral blood samples were obtained. 2-According to the mnufacturer's protocol, total DNA was purified using Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit . (Thermo Fisher Scientific, USA). 3-DNA amplification using PCR technique was performed using Thermo Scientific DreamTaq Green master. A PCR product of 156 base pairs (bp) was generated. 4. The amplified PCR products were digested with Mva1269I (BsmI) Restriction enzyme at 37°C for 1.5 hours. 5. The digested products were applied to gel electrophoresis in a 2% agarose gel (125V for 35 minutes). 6. Genotypes were determined as follows: KEL*02/KEL*02 (An undigested 156 bp band), KEL*01/KEL*02 (three bands (156 bp, 100 bp and 56 bp))

Results

Table (1):Kell1 antigen phenotyping in transfused thalassemic patients

Kell1 antigen phenotyping	No.	%
K1 (+)	2	3.3
K1 (-)	43	71.7
Mixed field (Mf)	15	25.0

Expected genotype for K1 (-) phenotype is KEL*02/KEL*02 , while K1 (+) phenotype maybe KEL*01/KEL*02 or KEL*01/KEL*01 genotype

Table (2):Kell antigen genotyping in transfused thalassemic patients

Kell1 antigen genotyping	No.	%
KEL*01/KEL*02	4	6.7
KEL*02/KEL*02	56	93.3

Two out of the 60 patients showed discrepancies between genotyping and phenotyping results as shown in table 3

Table (3):Discrepancy of Kell1 antigen phenotyping versus genotyping in transfused thalassemic patients

Patient Number	Age	Sex	Age of diagnosis	Hb (gm/dl)	ABO phenotype	kell1 antigen phenotype	kell antigen genotype
1	7.5 yrs	Male	6m	7	O+	K1 (-)	KEL*01/KEL*02
2	5 yrs	Female	2.5m	6	B+	K1 (+)	KEL*02/KEL*02

Conclusion

The use of DNA-based antigen testing can significantly improve the effectiveness and reliability of blood group antigen typing and resolve undetermined blood group phenotypes in multi-transfused thalassemic patients .

The use of kell antigen genotyping combined with conventional serological assays can lead to a better antigen-matched blood transfusion as well as improvement of patient care.