Case Report

Use of the Gel Test to Follow Up Chimerism in ABO Mismatched Bone Marrow Transplantation Patient: A Case Report

Rachata Lumkul MD*, Oytip Nathalang PhD**, Pasra Arnutti MSc**, Aree Janyatham RN*, Kitti Torcharus MD*, Triroj Krutvecheo MD*, Thip Sriphaisal MD*

** Hematology-oncology Division, Department of Pediatrics, Phramongkutklao Hospital*
*** Department of Pathology, Phramongkutklao College of Medicine*

Objective: *The authors report here our experience using the gel test to follow up chimerism in a 5 year old girl with β thalassemia/Hemoglobin E disease (β thal/HbE), post allogeneic bone marrow transplantation with Hb E trait HLA identical sibling donor. They were ABO blood group major mismatched donor-recipient pairs (donor and recipient blood group are B and O, respectively).*

Material and Method: *Pre and post transplanted EDTA blood samples from the girl with β thalassemia/ Hemoglobin E were tested for ABO, Rh and direct antiglobulin test (DAT) using the A-B-AB-D-ctl/ AHG card and the titer of anti-A and anti-B were tested by the conventional tube technique. The sex chromosome study and hemoglobin typing were also examined.*

Results: *In this technique, mixed field agglutination is clearly identified from positive and negative results. The authors detected peripheral recovery, mixed O/B population after transplantation on day +26 with positive DAT. The DAT was negative on day +67 after transplantation and the recipient blood group was completely changed to B on day +123. In addition, Hb typing was changed to Hb E trait with Hb F less than 5 % on day +37. The engraftment of neutrophils, more than $5x10^9$/L, was detected on day+14 and platelet count was more than $20x10^9$/L on day +28. On day +90, the patient was transfusion-independent with the mean Hb level at 11.4 g/dL (10.4-13.1). The sex chromosome and hemoglobin typing were changed to the donor on day +300.*

Conclusion: *The gel test is an alternative method which is simple and helpful in detecting mixed red blood cell populations, particularly in the ABO or other blood group mismatched bone marrow transplantation.*

Keywords: *Gel test, Chimerism*

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Correspondence to: Lumkul R, Hematology-Oncology Division, Department of Pediatrics, Phramongkutklao Hospital, Bangkok 10400, Thailand. Phone: 0-2354-7600 ext. 94143, Fax: 0-2644-4130.

Allogeneic hematopoietic stem cell transplantation has been used to cure various hematological diseases, for instance, severe aplastic anemia, congenital immunodeficiencies and

thalassemia major. Many problems affect the result of transplantation such as recurrence of diseases, graft rejection, acute and chronic graft versus host diseases[1,2]. The term chimeras distinguish such patients to be complete chimeras, the only hematopoietic cells that can be detected are those of the donor and mixed chimeras, which is a mixture of donor and recipient hematopoietic cells. One of the most important issues is the method to evaluate the chimeras. Several genetic markers are used including DNA restriction fragment length polymorphisms and polymerase chain reaction, chromosomes, immunoglobulin allotypes and red blood cell (RBC) antigens[3-5]. The authors reported our experience using gel test in detecting chimerism in allogeneic bone marrow transplantation β-thalassemia/hemoglobin E (β-thal/HbE) patient. In this technique the positive and negative results are distinctly identified and the mixed field agglutination is clearly detected. After transplantation, the authors followed the ABO blood group periodically and the hemoglobin typing and sex chromosome study were performed.

Case Report

A 5-year-old girl with β-thalassemia/hemoglobin E (β-thal/HbE) was transplanted with hematopoietic stem cells from the bone marrow of Hb E trait HLA of an identical sibling male donor. They were ABO blood group major mismatched donor-recipient pairs (blood group B and O in donor and recipient, respectively).

The patient received conditioning regimens[6,7] consisting of busulfan(BU) 3.5 mg/kg/day on days -9, -8, -7, -6; cyclophosphamide (CY) 50 mg/kg/day on days -5, -4, -3, -2 with mesna 200 mg i.v. drip 15 minutes before cyclophosphamide then post 3, 6 and 9 hours. Marrow cells were collected on day 0 under general anesthesia, from the posterior iliac crest with a target marrow volume at 20 ml/kg recipient body weight. Total nucleated cell count (TNC) was obtained using an automated counter. The number of CD34+ cells was assessed by flow cytometry. Fresh and unmanipulated marrow was infused on the same day (day 0). With ABO major blood group incompatibility, red cells in the marrow were removed by sediment manipulation[6].

A combination of cyclosporinA (CsA) and methotrexate(MTX) was used for graft versus host disease(GVHD) prophylaxis at different stages of transplant course[6,7]. CsA was given at 3 mg/kg/day i.v.drip every 12 hours on day - 1 until gut function recovered, then changed to 12.5 mg/kg/day orally in three divided doses. CsA levels were measured weekly by fluorescent polarization immunoassay and the dose adjusted accordingly to maintain serum levels at approximately 200 ± 50 ng/ml. CsA dose was reduced if the trough level of CsA was over 300 ng/ml or serum creatinine level exceeded 2 mg/dl. MTX was given at 15 mg/m^2 on day +1 and 10 mg/m^2 on day +3, +6 and +11. Acute and chronic GVHD were graded and staged according to concensus conference on GVHD grading. Acute grade I GVHD or more than grade I was treated with methylprednisolone 1-2 mg/kg/day for 2 weeks and tapered as soon as possible.

The transplanted patient was hospitalized in a room with a high-efficiency particulate air filter until the neutrophils count recovered to $1x10^9$/l. She received prophylactic trimethoprim-sulfamethoxazole for pneumocystis carinii, ciprofloxacin for selective gut decontamination and fluconazole for fungal prophylaxis and acyclovir for Herpes simplex prophylaxis. Fever during the period of neutropenia was treated with broad-spectrum antibiotics. Intravenous immunoglobulins were given at a dose of 500 mg/kg weekly starting at day 1 posttransplantation. G-CSF 10 μg/kg/day was administered from the second day of transplant until the neutrophils count reached $0.5x10^9$/l or 3 consecutive days. Patients were transfused if their

Table 1. Cell grouping in βthal/HbE recipient mixed field agglutination (mf) of red cells were detected on day +26 post BMT and disappeared on day +123

Cell grouping	D-1	D+1	D+5	D+12	D+20	D+26	D+34
O	+	+	+	+	+	mf	mf
B	-	-	-	-	-	mf	mf

Cell grouping	D+37	D+44	D+49	D+54	D+58	D+67	D+123
O	mf	mf	mf	mf	mf	mf	-
B	mf	mf	mf	mf	mf	mf	+

+ : agglutination

- : unagglutination

mf : mixed field agglutination

hemoglobin or their platelets count was below 80 g/l or $20x10^9$/l, respectively.

For the evaluation of engraftment, daily complete blood count (CBC) was taken. Neutrophil engraftment was defined as the first day of ANC> $0.5x10^9$/l after the neutrophils nadir. Platelet engraftment was defined as the day when the platelet count exceeded $20x10^9$/l without previous platelet transfusions for at least 7 days. The donor engraftment and chimerism were evaluated by either cytogenetic (sex mismatch) and gel test for ABO-Rh(D) typing. Fifty microliters of 1% red blood cell suspension in ID-diluent 2 was added to each microtube of the ABO/Rh for newborns (with monoclonal antibodies, polyvalent antihuman globulin) card (DiaMed AG, Switzerland). The test card was centrifuged for 10 min using the present cycle of the centrifuge supplied for the system (ID- centrifuge 24S, DiaMed AG, Switzerland). The agglutination reactions were graded according to the distribution of the agglutinated particles throughout the gel matrix; negative reactions appeared as a discrete cell button at the base of the column, as indicated by the manufacturer[8]. Titer of anti-A and anti-B were also tested with standard A-cells and B-cells (National Blood Centre, Thai Red Cross Society, Thailand) by the conventional tube technique[9].

Results

The patient was infused with $4.88x10^6$/kg of CD34+ cell of Hb E trait identical to the sibling donor. She spent 45 days at the hospital and donor cells were engrafted on day +28 with blood component independence on day+90. There was no major complication except mild hypertension due to the side effect of CsA. A total of 14 blood samples of the patient before and after BMT were tested for ABO/RH typing by the gel test. The data of red blood cell grouping in the recipient (Table 1) showed a gradual change of blood grouping from group O of the recipient cells to group B of the donor cells on day +123. The mixed field agglutinations (mf), related to positive of direct antiglobulin test (DAT), were detected since day+26 and clearly identified from positive and negative results. The mf was still detected until day+67 and gradually disappeared on day+123 compatible with disappearance of DAT+ve (Fig.1 and 2). On day+300, Hemoglobin typing and sex chromosome study also changed to Hb E trait and 46XY of donor, respectively.

Discussion

In the present report, the authors described the use of gel test to detect chimerism in one allogeneic BMT patient. Although there are many

Fig. 1 Blood group and direct antiglobulin testing (DAT) by the gel test of O/B mismatched allogeneic BMT in βthal/HbE patient. A: Group O (Day-1 BMT), B: Group O/B, DAT+ve(Day+45 post BMT), C: Group B, DAT-ve (Day +123 post BMT)

methods to identify and quantify dual cell populations,[10,11] the gel test is one option in the case of mismatch ABO blood group in hematopoietic stem cell transplantation because it permits sensitive and specific reactions[12]. In contrast to other analysis for chimerism which need experienced personnel and complicated procedures such as chromosome analysis in case of sexual difference of donor and recipient, DNA analysis by restriction fragment length polymorphisms and the polymerase chain reaction. Fortunately, the authors could use gel test to follow up chimerism for patients, which have different blood groups. In this technique, mixed field agglutination is clearly identified from positive and negative reactions. A mixed ABO population was found on day +26 post BMT, whereas anti-B in the patient's serum was negative. A previous study reported the detection of a mixed ABO population on 9-15 days after transplantation in most cases of ABO mismatch bone marrow transplantation patients but delayed mixed field agglutinations were detected in minor blood groups mismatched with complication in case of graft failure and recurrence of malignant disease[12]. In the authors' experience, it was delayed detection of mixed field agglutination in the presented patient and DAT was positive on day +26 and negative on day +67. Because of the small sample the authors, were unable to find out the cause of difference in prolonged mixed field appearance in thalassemic patients. On day +123, the patient's blood group was completely changed to group B, hemoglobin typing was changed to Hb E trait similar to the donor. On day +90, the patient was transfusion independent with Hb 11.4 g/dL. Red blood cell phenotypes are highly informative genetic markers. Several antigen systems are frequently found to be different between donor and recipient. Further study using gel test to follow up chimerism in ABO mismatched transplanted thalassemic patients would be interesting and needs more samples to conclude the optimal time of recovery donor cells in recipient bone marrow.

Conclusion

The gel test is useful, cost effective and practical compared to other methods for detection of chimerism in hematopoietic stem cell transplanted

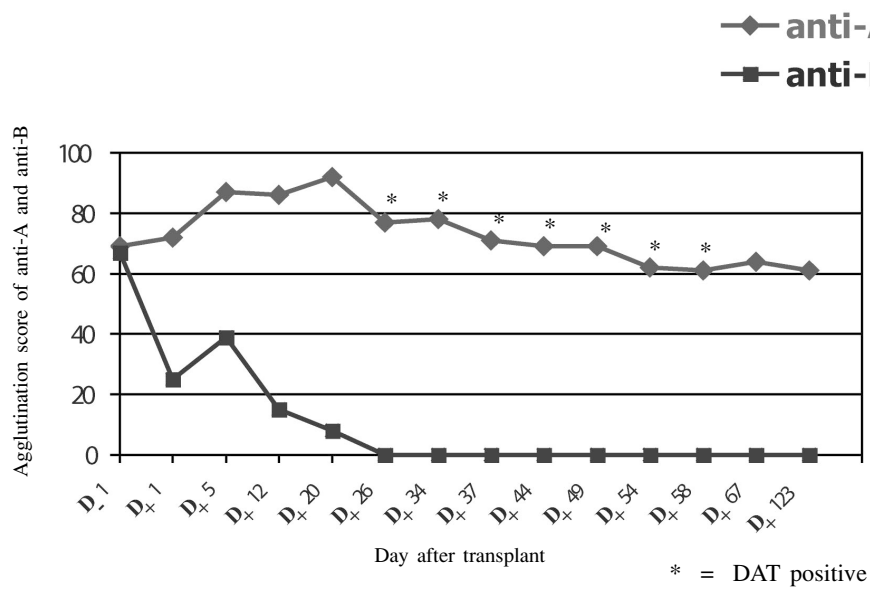

Fig. 2 Agglutination score of anti-A and anti-B in the recipient

patients that have ABO mismatched donor-recipient pairs. Physicians may select this method to follow chimerism in bone marrow transplanted recipients.

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รายงานการทดสอบแอนติเจนบนผิวเม็ดเลือดแดงด้วยวิธี gel test เพื่อติดตามการเจริญของเซลล์ผู้บริจาคในการปลูกถ่ายไขกระดูกของผู้ป่วยเด็กโรคโลหิตจางธาลัสซีเมีย

รัชฎะ ลำกูล, อ้อยทิพย์ ณ ถลาง, ภัสรา อาณัติ, อารีย์ จรรยาธรรม, กิตติ ต่อจรัส, ไตรโรจน์ ครุธเวโช, ทิพย์ ศรีไพศาล

วัตถุประสงค์: *รายงานการใช้การทดสอบแอนติเจนบนผิวเม็ดเลือดแดงด้วยวิธี gel test เพื่อติดตามการเปลี่ยนแปลงกลุ่มเลือดในผู้ป่วยเด็กหญิงไทยอายุ 5 ปี โรคโลหิตจางเบต้าธาลัสซีเมีย/ฮีโมโกลบินอี เลือดกรุ๊ปโอ ซึ่งได้รับการปลูกถ่ายเซลล์ไขกระดูกที่เข้ากันได้จากน้องชายที่เป็นพาหะธาลัสซีเมียชนิดฮีโมโกลบินอี เลือดกรุ๊ปบี*

วัสดุและวิธีการ: *ตรวจเลือดผู้ป่วยก่อนและภายหลังปลูกถ่ายไขกระดูกเป็นระยะเพื่อหากรุ๊ปเลือด ABO/Rh ตรวจ Direct Antiglobulin Test (DAT test) โดยวิธี A-B-AB-D-ctl/AHG card และตรวจหา anti-A anti-B titer รวมทั้งการศึกษาการเปลี่ยนแปลงกรุ๊ปเลือดของผู้ป่วยจากกรุ๊ปโอเป็นบี ตรวจโครโมโซมเพศและชนิดของฮีโมโกลบินที่เปลี่ยนแปลง*

ผลการศึกษา: *การเปลี่ยนแปลงกรุ๊ปเลือดเริ่มชัดเจนพบกรุ๊ปเลือดที่ผสมกัน (O/B mixed field agglutination) ในวันที่ 26 หลังปลูกถ่ายเซลล์ไขกระดูก รวมทั้งพบผลบวกจากการตรวจ DAT ซึ่งเปลี่ยนเป็นผลลบในวันที่ 67 กรุ๊ปเลือดผู้ป่วยเปลี่ยนเป็นกรุ๊ปเลือดของผู้บริจาคในวันที่ 123 พบนิวโทรฟิลมากกว่า 500 ตัวต่อไมโครลิตร ในวันที่ 14 และเกล็ดเลือดมากกว่า 20,000 ตัวต่อไมโครลิตร ในวันที่ 28 หลังปลูกถ่ายเซลล์ไขกระดูก ผู้ป่วยมีค่าความเข้มข้นของฮีโมโกลบินประมาณ 11.5 กรัมต่อเดซิลิตรโดยไม่ต้องการการให้เลือดตั้งแต่วันที่ 90 หลังปลูกถ่ายไขกระดูก รวมทั้งโครโมโซมเพศและชนิดของฮีโมโกลบินเปลี่ยนเป็นของผู้บริจาคในวันที่ 300*

สรุป: *การตรวจหาการเปลี่ยนแปลงกรุ๊ปเลือดเพื่อติดตามการเจริญของเซลล์ผู้บริจาคภายหลังการปลูกถ่ายไขกระดูกด้วยวิธี gel test เป็นอีกทางเลือกหนึ่งที่สะดวกประหยัดและง่ายโดยเฉพาะผู้ป่วยที่มีกรุ๊ปเลือดที่แตกต่างกับผู้บริจาค*