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Successful cord blood transplantation in thalassemia major patient at King Chulalongkorn Memorial Hospital

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In Thailand, homozygous β-thalassemia and β-thalassemia/hemoglobin E diseases, so-called thalassemia majors, are the most common and the most severe clinical syndromes compatible with live birth. Allogeneic hematopoietic stem cell transplantation is accepted as the only curative treatment for thalassemia major. Sources of donor stem cells can be achieved from bone marrow, peripheral blood, or placental and umbilical cord blood. Umbilical cord blood units have been globally used as alternative source of stem cells for transplantation into thalassemic patients. Here we report the first successful HLA-matched related umbilical cord blood transplantation in a boy with β-thalassemia/hemoglobin E disease from his heterozygous hemoglobin E newborn brother at King Chulalongkorn Memorial Hospital.

Keywords: *Cord blood transplantation, Thalassemia.*

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ปรีดา วาณิชยเศรษฐกุล, ธีระ วัชรปรีชานนท์, ปราณี สุจริตจันทร์, ปัญญา เสกสรรค์, รัชนี โอเจริญ. การปลูกถ่ายเลือดจากสายสะดือสำเร็จในผู้ป่วยโรคโลหิตจางธาลัสซีเมียชนิดรุนแรง. จุฬาลงกรณ์เวชสาร 2546 ก.ค; 47(7): 411 – 8

โรคโลหิตจางธาลัสซีเมียที่รุนแรงชนิดโฮโมซัยกัสเบต้าธาลัสซีเมียและเบต้าธาลัสซีเมียฮีโมโกลบินอีเป็นโรคที่พบได้บ่อยมากในเด็กไทย การปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตจากผู้บริจาคได้รับการยอมรับในวงการแพทย์ว่าเป็นวิธีการเดียวในปัจจุบันที่สามารถรักษาโรคธาลัสซีเมียชนิดรุนแรงให้หายขาด แหล่งของเซลล์ต้นกำเนิดเม็ดโลหิตหามาได้จากไขกระดูก กระแสเลือด หรือเลือดจากสายสะดือและรกของเด็กทารกแรกเกิด ในสถาบันการแพทย์ทั่วโลกรวมทั้งบางสถาบันในประเทศได้มีการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตเพื่อรักษาผู้ป่วยเด็กโรคโลหิตจางธาลัสซีเมียมาแล้วหลายราย รายงานนี้เพื่อนำเสนอผลการปลูกถ่ายเลือดจากสายสะดือสำเร็จในผู้ป่วยเด็กโรคเบต้าธาลัสซีเมียฮีโมโกลบินอีโดยใช้เลือดจากสายสะดือและรกของน้องชายที่เป็นทารกแรกเกิดที่มี HLA ตรงกัน และเป็นแค่พาหะของฮีโมโกลบินอี นับเป็นรายแรกของโรงพยาบาลจุฬาลงกรณ์

Hematopoietic stem cell transplantation is currently the only curative treatment for thalassemia major.[1-3] However, less than 30 percent of the patients have unaffected HLA-identical siblings to serve as donors. Umbilical cord blood contains substantial numbers of hematopoietic stem cells and has been utilized worldwide as alternative source of stem cells for transplantation into patients with Fanconi's anemia, aplastic anemia, leukemia, congenital immunodeficiencies, thalassemia, and others.[4-13] We report the first successful transplantation of cord blood stem cells from an unaffected, HLA-identical sibling to a boy with β-thalassemia/hemoglobin E disease at our institute.

Case Report

A 3 1/2-year-old boy was first detected anemic at 1 year and 10 months of age. He was given a diagnosis of β-thalassemia/hemoglobin E disease. His initial hemoglobin level was 5.8 g per deciliter, the mean corpuscular volume was 60.8 fl, and reticulocyte count was 5.1 percent. His hemoglobin electrophoresis revealed hemoglobin E and F (33.53 percent and 49.20 percent, respectively). Typing of the boy's father revealed that he was heterozygous for hemoglobin E (hemoglobin E, 28 percent; hemoglobin A, 72 percent); typing of his mother showed that she was heterozygous for β-thalassemia (hemoglobin A, 93.1 percent; hemoglobin A_2, 4.9 percent; hemoglobin F, 1.81 percent). The patient had received 20 units of leukocyte-filtered packed red cells at 3 to 4 weeks intervals and had been treated with iron chelation. He had slight hepatosplenomegaly. Initially, bone marrow transplantation had not been considered because his only elder sister, who was heterozygous for β-thalassemia, did not have HLA-identical with him.

However, the patient's mother subsequently became pregnant in April 2001. Prenatal tests for thalassemia were performed in August 2001. DNA extracted from chorionic villi sampling was tested and proved that the fetus was heterozygous for hemoglobin E and no β-thalassemia mutation was found. The pregnancy was then continued. Cordocentesis was done in September 2001 and the result of in-utero cord blood indicated that the fetus gender was male and the HLA of the fetus was identical to that of the patient. Their HLA types were A *11; B *1501, *5201 (Bw 4, 6); DRB1 *04, *15; DRB4 *01; DRB5 *01-*02. The ABO blood groups were type A and AB in the patient and his brother, respectively. Hence, the delivery process was scheduled in advance for cesarean section in January 2003, in order to facilitate cord blood collection for optimal yield.

A boy was delivered without any complication on January 4, 2002. He was a term neonate of 37 gestational age with birth weight 2,800 gram. Ninety-milliliter volume of cord blood was aseptically collected immediately after the baby was born but before placenta delivery, in acid citrate dextrose filled bag. A portion of unclotted cord blood was spared for infectious markers screening test and cell count. Then the main volume of cord blood was transferred to a freezing bag, mixed with dimethylsulfoxide in minimal essential medium with no separation, and cooled in a controlled-rate freezer before being frozen in liquid nitrogen. The eventual volume of cryopreserved cord blood was 80 milliliter. The infectious markers of cord blood showed negative HBsAg, HCV-Ag, anti-HIV, VDRL, anti-CMV IgM but

positive anti-CMV IgG (7.72 IU per milliliter). Bacterial culture results showed no growth of either aerobe or anaerobe. The number of total CD 34+ cells preserved were 0.44 X 10^6 that were calculated as 0.26 X 10^5 cells per kilogram body weight of recipient because the patient's weight was 17 kilograms.

Pre-transplant evaluation for the patient revealed normal echocardiogram, normal pulmonary function test, normal audiogram, but the result of liver biopsy showed non-specific minimal liver damage with mild fibrosis and hemosiderosis. The serum ferritin level was 925 ng per milliliter. Infectious markers studies revealed negative anti-HIV, positive anti-CMV IgG but negative IgM, positive anti-HSV IgG but negative IgM, positive anti-EBV IgG but negative IgM, hepatitis A, B, C profiles were all negative. The patient was assessed as Lucarelli's class 2. Prior to receiving the cord blood, he was inserted double-lumen central venous catheter. Thereafter, he was conditioned with busulfan, cyclophosphamide, and equine anti-thymocye globulin (ATG).[1-3] The dose of busulfan was 4 mg per kilogram per day given for 4 days, from day -9 to day -6. The dose of cyclophosphamide was 50 mg per kilogram per day given for 4 days, and equine ATG was given 20 mg per kilogram per day for 4 days, both from day -5 to day -2.

On February 14, 2002, the frozen cord blood was thawed in water bath at 37°C and immediately given as intravenous infusion over 25 minutes into the patient. 79 percent of stem cells in the thawed cord blood were viable. Intravenous cyclosporine was given as prophylaxis against graft-versus-host disease (GvHD). Granulocyte colony-stimulating factor (G-CSF) was injected intravenously, 5 microgram per kilogram per day started since day 1 after cord blood infusion, and continued until the absolute neutrophil count remained above 2,000 cells per cubic millimeter for 2 consecutive days.

The patient's clinical course after cord blood stem cell transplantation was mildly eventful. Febrile neutropenia developed on day 6 without demonstrable source of infections. Cloxacillin and ceftazidime were then administrated. Two days later the fever subsided and positive urine culture for klebsiella species sensitive to ceftazidime was reported. However, his blood culture was never positive. On day 13 low-grade fever with diffused fine erythematous rashes appeared which were interpreted as serum sickness reaction. No specific treatment was given. The rashes and fever subsided on their own in next few days. On day 15, clinically mild hepatic veno-occlusive disease was suspected due to increment of body weight, presence of ascites, and hyperbilirubinemia. Supportive therapy was given and those abnormalities were improved in a week. The antibiotics were discontinued on day 25 after his neutrophil recovery and repeated urine culture revealed no growth.

The white blood cell count rose above 1,000 cells per cubic millimeter on day 22, and above 3,000 cells per cubic millimeter on day 38. The absolute neutrophil count rose above 500 cells per cubic millimeter on day 22 and exceeded 1,000 cells per cubic millimeter by itself, after quitting G-CSF injection on day 43. The platelet count exceeded 20,000 per cubic millimeter on day 46, and exceeded 100,000 cells per cubic millimeter on day 56. The patient received 8 units of leukocyte-filtered, irradiated packed red cells; the last of which was given on day 37. Leukocyte-filtered, irradiated, single donor platelet transfusions were given 14 times after transplantation;

the last one was given on day 36.

On day 48 after transplantation the patient was discharged home. The hemoglobin level was 9.3 g per deciliter; hemoglobin types A and A_2 were present. On day 63, during the follow-up, the hemoglobin level was 10.8 g per deciliter and it was noted that there were no longer enlarged liver and spleen. On day 105, the hemoglobin level was 12.4 g per deciliter (6.07 percent hemoglobin A_2, 28 percent hemoglobin F, and 65.93 percent hemoglobin A). On day 123, the hemoglobin level was 13.2 g per deciliter (11.98 percent hemoglobin E, 44.12 percent hemoglobin F, and 43.9 percent hemoglobin A). At 6 months, the hemoglobin level was 12.1 g per deciliter (10.17 percent hemoglobin E, 31.3 percent hemoglobin F, and 58.53 percent hemoglobin A); reticulocyte count was 0.2 percent; and the blood group was completely changed to AB as of donor. Recently at 1 year post-transplant, the patient was clinically healthy. He was attending kindergarten, and never had any features of GvHD. The hemoglobin level was 13.8 g per deciliter.

Engraftment was documented by chimerism analysis using microsatellite technique. Identified by marker D8S1179, there were signals at 12,13 of donor blood sample while there were signals at 15,15 of pre-transplant recipient blood sample. On day 47 post-transplant, there were signals at 12,13,15 of recipient blood sample by the same marker that indicated mixed donor-recipient chimerism. At 6 months post-transplant the 3 signals remained present but there were more prominent signals at 12,13 than at 15. It implied that the mixed chimera tended to have donor cells overgrowing recipient cells.

Discussion

Thalassemias and hemoglobinopathies have high prevalence in Thailand.[14] The frequency of α-thalassemia reaches 20 to 30 percent, whereas β-thalassemia occurs at a frequency of 3 to 9 percent. Hemoglobin E, the hallmark hemoglobinopathy of Southeast Asia, occurs at a frequency of 13 to 50 percent. Mutation and gene interaction account for more than 60 different clinical syndromes. Among these, homozygous β-thalassemia and β-thalassemia/hemoglobin E diseases are the most common and the most severe clinical syndromes compatible with live birth.[15,16] These are so-called thalassemia major. β-thalassemia/hemoglobin E disease is more frequent than homozygous β-thalassemia in Thailand due to much a higher incidence of hemoglobin E.

Transplantation of hematopoietic stem cells from HLA-identical siblings offers a high possibility of cure in patients with thalassemia major, particularly those in Lucarelli's class 1 or 2. The disease-free survivals are 94 and 77 percent in patients class 1 and 2, respectively.[17,18] Patients who undergo transplantation early in the course of thalassemia, or receive regular hypertransfusion with adequate iron chelation therapy, have higher chance of cure and less complication. In clinical practice, however, the severity of the disease would be determined on the basis of genotype, and stem cell transplantation should be performed as soon as possible in children with severe thalassemia. Sources of stem cells can be achieved from donor's bone marrow, peripheral blood stem cells, or placental and umbilical cord blood. There have been more than 1,000 thalassemia patients who underwent stem cell transplantation

worldwide.[19,20] In Thailand there have been more than 100 β-thalassemia major patients who underwent stem cell transplantation. Among these there were more than 16 cases of related cord blood transplant.[21-25] However, for the first time at King Chulalongkorn Memorial Hospital we experienced a successful cord blood transplantation for thalassemia.

The engraftment rate after cord blood transplantation in our patient was slower than would be expected after bone marrow transplantation. The absolute neutrophil count reached 500 cells per cubic millimeter by day 22, and the platelet count exceeded 20,000 per cubic millimeter by day 46, despite the administration of G-CSF after transplantation. The corresponding values for the time to hematologic recovery after bone marrow transplantation for thalassemia are usually 18 days for absolute neutrophil count and 21 days for platelet count. Apparently, the engraftment rate in our patient was similar to that in other patients who received cord blood transplantation.Previous studies showed that chimerism was common after bone marrow transplantation for thalassemia. We found mixed donor-recipient chimerism in our patient on day 47 that persisted for 6 months. It revealed a tendency of donor chimera increasing dominance over recipient chimera. We expect the more donor predominance from the result of one-year study that is pending at the report time. The presence of considerable amount of hemoglobin F early post-transplant might represent physiologically high fetal hemoglobin content in normal cord blood, and hemoglobin F amount appeared to go down from day 123 to at 6 months. We expect its lesser amount at one-year post-transplant follow-up hemoglobin typing.

Conclusion

Here we report the first successful HLA-matched related umbilical cord blood transplantation in a boy with β-thalassemia/hemoglobin E disease from his heterozygous hemoglobin E brother. Currently, it is one-year post transplant. He has been doing well, and has normal hemoglobin level without any need for blood transfusion. The recent engraftment study revealed mixed chimera with donor predominance. The outcome of this transplant is good and comparable with experiences of other hospitals.

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