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Chimerism in pediatric hematopoietic stem cell transplantation and its correlation with the clinical outcome

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is the only hope to cure many inherited and acquired hematological disorders in children. Monitoring of chimerism helps to predict the post-transplantation events, with the intention to enhance the long-term disease free survival (DFS). The study aimed to investigate the importance of early chimerism detection to predict the clinical outcome following HSCT. The study included nine recipients (six β-thalassemia and three severe aplastic anemia patients) and their 10/10 HLA identical sibling donors. Chimerism detection was performed by analysis of short tandem repeat (STR) polymerase chain reaction (PCR) for detection and quantification of the relative amounts of donor and recipient cells present on day + 28. Peripheral blood (PB) was the main stem cell source for HSC transplantation. Disease free survival (DFS) was 71.4% while overall survival was 85.7% for PBSC transplants at the median follow up period of 4 years. The early detection of chimerism by PCR-STR analysis for children with β-thalassemia and aplastic anemia correlated with the outcome of HSCT in 8 (88.8%) patients. Complete chimerism was associated with disease-free survival while mixed chimerism and autologous patterns were associated with poor prognosis. In conclusion, early chimerism testing is clinically important in prediction of outcome after allogeneic HSC transplantation.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only approach for many non malignant hematological diseases in children [1]. In Egypt, the most common type of the hemoglobinopathies is β-thalassemia major (BTM), a disease usually associated with lower health related quality of life [2,3]. Since the prospect of gene therapy is limited to developed countries, allogeneic HSCT remains the only available definitive cure for children with this severe inherited disease in deficient resources. HSCT replaces the ineffective erythropoiesis associated with the disease with an allogeneic stem cell capable of effective erythropoiesis. Children suffering from transfusion dependent β-thalassemia, who underwent HSCT, experienced higher health-related quality of life than patients treated with transfusion and chelation [4].

Human leukocyte antigen (HLA) compatible stem cells from an unaffected identical sibling are regarded as the best strategy for β-thalassemia patients requiring allogeneic transplantation. Improving the clinical outcome among patients receiving allogeneic HSCT is considered a challenge and progressively changes practice [5]. Bone marrow (BM) has been the preferred source of stem cells in β-thalassemia, however, a shift to peripheral blood stem cells (PBSC) has been suggested to prevent graft failure, for the potential of the graft-letting effect of the T-cell content of PBSC grafts. The PBSC transplants, despite providing faster engraftment and immune reconstitution, are associated with an increased incidence of graft versus host disease (GVHD), a serious complication of HSCT, and a lower 2-year survival than BM grafts [6].

There also has been an expanding increase in the use of peripheral blood (PB) as an alternative source of hematopoietic stem cells for severe aplastic anemia (SAA) especially in countries with limited resources [7]. In the Eastern Mediterranean region, the hemoglobinopathies accounted for 7% of HSCT while bone marrow failure syndromes comprised 12.2% of all HSCT performed [8]. SAA is an acquired and potentially fatal disease and HSCT from an HLA-matched sibling represents the initial treatment of choice for children with SAA.

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When treating patients at high risk of graft failure and infective complications, the utilization of PB is sometimes favored to promote early engraftment and independence from platelet and red cell transfusions. Nonetheless, a significantly inferior outcome was noted after PBSC transplantation, mainly due to the increased morbidity risk of GVHD as well as a lower 2-year survival [9].

The need for HSCT persists to rise, and out of necessity the improvement in the post transplant management continues. Surveillance of chimerism and the quantitative determination of donor-specific cells in the recipient have become critical for predicting the success of HSCT [10]. Complete donor derived hematopoiesis is crucial for maintenance of engraftment and prevention of recurrence of the underlying disease [11]. An accurate quantitative analysis of chimerism would allow early identification of patients with a high risk of graft versus host disease or those liable to disease relapse in addition to early differentiation between failure of engraftment and delay in engraftment [12].

2. Objective

The objective of our work was the early detection of chimerism after allogeneic HSCT in β-thalassemia and SAA in pediatric patients and the correlation of the results with the clinical outcome. Another purpose was to compare between short tandem repeat (STR) analysis and variable number tandem repeat (VNTR) analysis for detection of chimerism in the patients.

3. Materials and methods

This is a study of allogeneic HSCT for children with severe transfusion dependent β-thalassemia and severe aplastic anemia. The study included 6 β-thalassemia major class 2 and 3 SAA patients who underwent HSCT from their siblings at the Hematology and Bone marrow transplantation Unit in Cairo university Aboualreesh Monerrah pediatric hospital. The study was approved by the research ethics committee of Cairo University. Parental informed consents were obtained from the parents and assent forms from the donors and recipients were collected.

3.1. Patients and their respective donors

The donors of HSCT were HLA matched siblings. Patients with severe β-thalassemia were confirmed by hemoglobin electrophoresis, high-performance liquid chromatography, DNA testing and bone marrow examination. Their sibling donors underwent hemoglobin electrophoresis and bone marrow examination. For SAA patients and their HLA identical donors, chromosomal breakage studies were performed. Laboratory investigations included serum ferritin, screening for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Toxoplasma. ABO and Rhesus (RH) blood grouping, and HLA typing were tested. HLA types were determined using polymerase chain reaction (PCR) followed by hybridization with a panel of biotinylated sequence-specific oligonucleotide probes (INNO-LiPA, Fujirebio). Chest X ray, abdominal and pelvic ultrasound, echocardiography and dental consultation were performed for all patients.

The patients were hospitalized 12 days before transplantation in β-thalassemia and 6 days in aplastic anemia. The patients received the conditioning regimen according to their diagnosis. In β-thalassemia, the protocol comprised buslfan 16 mg/kg/total dose on day -7 to day -4, antithymocyte globulin 11 mg/kg/day between days -5 and -1 and days +1 and +5, cyclophosphamide 30 mg/kg/day on day -5 to day -2, dexamethasone 0.2 mg/kg/day on day -12 to day 0, methylprednisolone 2 mg/kg/day between days -7 and +4 then the dose was reduced 50% every week, ciclosporin A 3 mg/kg/day on day -1 for 1 year after transplantation. In SAA, the preparative regimen was cyclophosphamide 50 mg/kg/day for 4 consecutive days on day -5 to day -2, fludarabine 120 mg/m^2 total dose on day -3 to day -1, dexamethasone 0.2 mg/kg/day between days -5 and 0, ciclosporin A 3 mg/kg/day on day -1 for 1 year after transplantation. Only one SAA patient (Patient 1) received total body irradiation (TBI) in a total dose of 10Gy on 5 fractions in 2 fields on day -9 to day -5.

3.2. Hematopoietic stem cell harvest from the donors

Five β-thalassemia patients and 2 SAA patients received peripheral blood stem cells (PBSC). One SAA patient received bone marrow stem cell transplant (BMT) while 1 β-thalassemia patient received umbilical cord blood (UCB) stem cell transplant. In PBSC transplantation, stem cell mobilization in the donors by granulocyte colony stimulating factor (G-CSF, 10 μg/kg) subcutaneous injections for 3–5 days was followed by leukapheresis (COBE Spectra Apheresis System, Cobe Laboratories; Lakewood, CO). The cells were infused immediately after harvesting. For BMT, the donors received G-CSF (10 μg/kg) for 3 days, then bone marrow was collected from the posterior iliac crests under general anesthesia and infused immediately after harvesting. In umbilical cord blood transplantation, 50 ml of fetal cord blood were collected immediately after the cord was cut, into a blood collection set containing citrate phosphate dextrose anticoagulant. The unit was cryopreserved and stored in cord blood bank, then thawed and infused on the day of transplantation. Red cells were depleted by centrifugation because of major ABO mismatch.

3.3. Estimation of CD 34 + stem cell dose

The concentration of CD34 + stem cells was measured based on four-parameter flow cytometry (CD45-PerCP/CD34-PE staining, side and forward angle light scatter) according to ISHAGE protocol by the International Society of Hematotherapy and Graft Engineering [13]. This allowed the verification of CD34 + cells as being dim for CD45 fluorescence and having low side scatter [14]. In the lymphocyte and monocyte gate, the CD34 + stem cell dose was calculated (CD34 + cells % × lymphocytes % × total white blood cell count × total volume of the isolated mononuclear cells).

3.4. Detection of engraftment and clinical follow-up of the recipients

Major outcomes including engraftment and transplant-related complications such as infections, acute and chronic graft versus host disease (aGVHD, cGVHD, respectively), and death were recorded. Recipients received G-CSF (10 μg/kg) daily subcutaneous injections on day +6 until the total WBC count reached ≥ 1000 cells/cmm. The myeloid engraftment day was defined as the first of 3 consecutive days after transplantation in which the absolute neutrophil count was at least 500/cmm [15]. The patients received prophylaxis against infections with acyclovir 1500 mg/m^2/day and fluconazole 6 mg/kg/day. CMV reactivation was defined as detection of the virus by PCR. All transplant recipients received prophylaxis against GVHD by cyclosporine A 3 mg/kg/day. Diagnosis and grading of GVHD was performed according to consensus criteria [16].

3.5. Detection of chimerism by short tandem repeat (STR)-fragment length analysis

3.5.1. DNA extraction

Peripheral blood samples (2 ml) were collected in sterile EDTA vacutainers from donors and recipients before HSCT and on day +28 from the recipients. DNA was extracted according to manufacturer instructions (High pure PCR Template preparation kit, Roche Diagnostics, Germany). The extracted DNA was stored at -80 °C until further analysis.