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Chimerism Monitoring of Pediatric Oncology Patients After Hematopoietic Stem Cell Transplantation Using STR Analysis

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Introduction

Hematopoietic stem cell transplantation (HSCT) is becoming an increasingly important approach to treatment children and adults with different malignant and nonmalignant diseases (1). Donor chimerism analysis has become a routine method for the following the newly developed hematopoietic system is of recipient or donor origin (2).

This study aimed to evaluate the donor chimerism status using polymerase chain reaction (PCR) of short tandem repeat (STR) in pediatric patients with different malignant and nonmalignant diseases.

Methods

Our study includes 96 children with malignant and nonmalignant disorders. Twenty-six were malignant (ALL, AML, KML) and 70 were nonmalignant (thalassemia, sickle cell disease, immunodeficiency diseases, osteopetrosis, severe aplastic anemia, etc.) Consent form was taken from the patients. The patients underwent transplantation at Balcalı Hospital Bone Marrow Transplant Clinic. Fifthteen short tandem repeat alleles and Amelogenin gene of donor and recipients analyzed. The PCR reaction is set up as described in the AmpFlSTR® Identifiler® PCR Amplification Kit user manual (ABI) in a final volume of 25 mL: master-mix preparation 10.5 mL PCR buffer, 0.5 mL AmpliTaq Gold DNA polymerase and 5.5 mL of primer mix per sample. Fifteen µL of master-mix are added to 10 mL template DNA.

Chimerism status were determined by capillary electrophoresis of STRs. STR-based analyses were used before HSCT to determine the informative alelles and after for monitoring post-transplant chimerism (Figure 1).

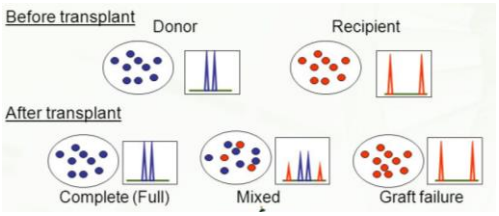


Figure 1. Two parts of chimerism analysis. Before (Pre) transplant **informative** analysis, After (post) transplant **engraftment** analysis.

Results

The patients with malignant disorders (26), who underwent HSCT, post-transplant (30th day and 60th day period) monitoring results were 46% complete donor chimerism, 19% mix chimerism and 15% graft rejection or nonengraftment. The patients with nonmalignant disorders (70) chimeric status were 59% complete donor chimerism, 19% mix chimerism and 14% graft rejection or nonengraftment (Figure 2).

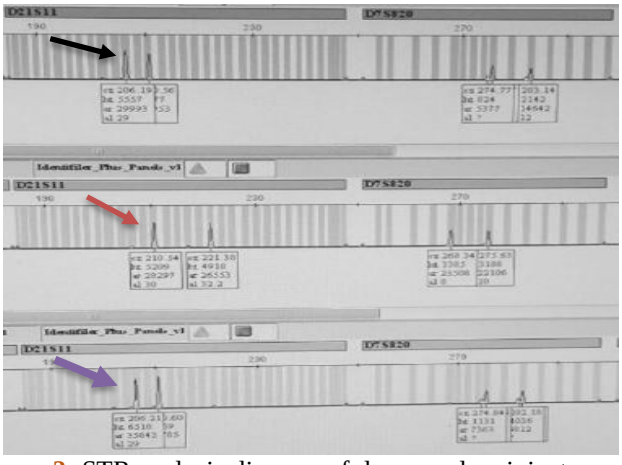


Figure 2. STR analysis diagram of donor and recipient samples. Before and after HSCT. The black arrow is donor; red arrow is recipient before transplant; the purple arrow is recipient 30 day after transplant (complete donor chimerism)

Discussion

Quantitative monitoring of recipient and donor-derived cells by molecular methods has become an indispensable diagnostic tool in the surveillance of patients undergoing allogeneic HSCT (3). STR analysis has been performed over a large area to monitor chimerism situation after HSCT using comerically available kits (4).

The patient/donor cell chimerism in this study reveals donor and recipient information during the post-transplant period for preemptive therapeutic interventions for clinicians.

Conclusion

Analysis of short tandem repeats (STR) is the predominant method for post-transplant monitoring of donor engraftment (5). Using STR–PCR-based serial analysis of microsatellite regions in short time intervals, it could be shown that patients with rapidly increasing mixed chimerism have the highest risk of relapse.

Recent Publications

1. Mahmoud Aljurf M, Abalkhail H, Alseraihy A et al., Chimerism Analysis of Cell-Free DNA in Patients Treated with Hematopoietic Stem Cell Transplantation May Predict Early Relapse in Patients with Hematologic Malignancies. Biotechnology Research International Volume 2016; 1-6.
2. Kletzel M, Huang W, Olszewski M, Khan S. Validation of chimerism in pediatric recipients of allogeneic hematopoietic stem cell transplantation (HSCT) a comparison between two methods. Chimerism 2013; 4(1): 1–8.
3. Biotechnical Methods Section BTS, Appendix: Method in Focus Leukemia volume15, pages303–306 (2001) doi:10.1038/sj.leu.2402008.
4. Han E, Kim M, Kim Y. et al. Practical informativeness of short tandem repeat loci for chimerism analysis in hematopoietic stem cell transplantation. Clinica Chimica Acta 2017; 468: 51-59.
5. Jordan R. Clark JR, Scott SD, Jack AL. et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. British Journal of Haematology 2014; doi:10.1111/bjh.13073.