# Relationship between Molecular Chimerism and Graft Versus Host Disease after Allogenic Hematopoietic Stem Cell Transplantation

Sare Etemad MD<sup>1</sup>, Hossein Ayatollahi MD<sup>2,\*</sup>, Maryam Salehi MD<sup>3</sup>, Payam Siyadat MD<sup>4</sup>, Nafiseh Amini MD<sup>1</sup>, Maryam Sheikhi MD<sup>1</sup>, Ali Fani MD<sup>5</sup>, Ali Ghasemi MD<sup>6,\*</sup>

- 1. Resident of Pathology, Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
- 2. Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
- 3. Qaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
- 4. Department of Hematology, School of Allied Medical Sciences, IRAN University of Medical Sciences, Tehran, Iran.
- 5. Radiologist, Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
- 6. Associate Professor of Pediatric Hematology and Oncology, Faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

\*Corresponding author: Dr.Hossein Ayatollahi, Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran Email:Ayatollahih@mums.ac.ir . ORCID ID: 0000-0003-4468-5884, Dr Ali Ghasemi, Associate Professor of Pediatric Hematology and Oncology, Faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Email: ghasemia1@mums.ac.ir ORCID ID: 0000-0003-4581-9362.

**Received:** 25 June 2019 **Accepted:** 10 December 2019

#### **Abstract**

**Background:** Bone marrow transplantation (BMT) is considered as a curative therapy for a broad range of diseases. However, complications such as relapse and graft versus host disease (GVHD) may be observed following BMT. Chimerism analysis serves as a reliable indicator of transplant outcome. Complete chimerism refers to the complete replacement of hematopoietic system by donor cells, while mixed chimerism is the coexistence of both donor and recipient cells. The current study aimed to assess the relationship between molecular chimerism and GVHD as well as relapse and survival after allogenic hematopoietic stem cell transplantation (allo-HSCT) using Short Tandem Repeat-Polymerase Chain Reaction (STR-PCR).

**Material and Methods:** This retrospective survival study was performed on 30 patients (Median age: 11.57±6.83 years) including 12 (40%) children with acute leukemia (6 patients (50%) acute myeloid leukemia and 6 patients (50%) with acute lymphoblastic leukemia patients). All patients received allo-HSCT during 2012-2016 at Montaserie Hospital, Mashhad, Iran. Chimerism analysis by STR-PCR method was carried out at cancer molecular pathology research center of Qaem hospital, Mashhad, Iran. Chimerism was assessed using 7-STR markers on recipients' bone marrow aspiration samples on day 14 or 15 after BMT.

**Results:** The findings indicated that the mean chimerism level in patients with skin GVHD was significantly different compared to cases without skin GVHD (P=0.02). It was also found that patients' survival was significantly longer in cases with complete chimerism (P=0.04).

**Conclusion:** Chimerism analysis may permit early prediction and monitoring of post-transplant complications such as GVHD, transplant rejection, and relapse and assist clinicians to proceed with suitable treatment plans.

Keywords: Acute leukemia, Chimerism, Graft-Versus-Host-Disease

#### Introduction

Bone marrow transplantation (BMT) is currently a curative therapy for many diseases, particularly acute leukemia (1). The major causes of BMT failure include graft rejection and relapse of the underlying disease. Follow-up of transplanted recipients is a crucial measure to early predict post-transplant adverse

events such as relapse, transplantation rejection, and graft versus host disease (GVHD) which can negatively affect treatment outcome (2-4). GVHD is a major cause of death and inability among transplant recipients and is defined as the recipient's immune response against transplanted tissues. Skin, liver, and digestive tract are three commonly

affected tissues by GVHD (5-10). When the signs of GVHD appear 100 days after transplantation, it is classified as chronic GVHD (10). It would be feasible to prevent GVHD before the emergence of clinical signs by providing treatments such as immunosuppressant agents and prophylactic donor lymphocyte injection (DLI) particularly in patients with a high possibility of future relapse (6-8).

It has been proposed that chimerism can monitoring after BMT provide information valuable that enables clinicians to early detect disease relapse facilitates **GVHD** and further therapeutic interventions. The term chimerism refers to the presence of hematopoietic cells of donor origin after bone marrow transplantation. complete chimerism is considered as a complete replacement of a patient's hematopoietic system with donor cells (1). However, mixed chimerism is described as a state in which the recipient's lymphohematopoietic system consists of a mixture of both donor and recipient's cells (12-15). Mixed chimerism is assessed to detect post-transplant relapse and to predict engraftment success (4). The presence of less than 5% of donor's cells is defined as lack of chimerism or transplantation rejection phenomenon (1, 16).

Nowadays, molecular genetic methods which can efficiently differentiate donor and recipient's cells are used in routine clinical practice. STR-PCR is a commonly used and highly sensitive method for chimerism quantification Chimerism analysis takes advantage of polymorphic short tandem repeat markers within the genome to discriminate donor recipient from **DNA** (2-4).polymorphic nature of STR regions facilitates the discrimination between the donor and recipient DNA profile. One of the advantages of STR-PCR is that it can identify multiple STR loci simultaneously. The detection of both donor and recipient STR loci is an indicator of the presence of

recipient and donor cells. The relative amount of each identified STR locus shows the percentage of chimerism in the patient's post-transplant specimen. In addition, elevated and persistent levels of recipient cells could be an indicator of graft rejection or relapse (16-18).

Short tandem repeat - polymerase chain reaction (STR-PCR ) is a widely-used method with a detection limit of 5%, which is considered as the base for classifying chimerism into complete chimerism (95 %≤) and lack of chimerism (5%>) (19). The objective of the present study was to further elucidate the association between chimerism analysis by STR-PCR and factors such as survival, acute graft versus host disease (aGVHD), and chronic graft versus host disease (cGVHD) in patients who received allogenic bone marrow transplantation.

# **Materials and Methods**

The current study was a retrospective survival analysis on 30 patients who were transplanted at blood and oncology research centre of Montaserieh Hospital 2012-2016. study during This approved by the Ethics committee of Mashhad University of Medical Sciences (Ethical code: T 4482).Patients' clinical information was extracted from archived medical records. The chimerism analysis was carried out at molecular pathology and cytogenetic center of Oaem Hospital, Mashhad, Iran, on day 14 or 15 after BMT. All included patients were qualified for hematopoietic allogenic stem cell transplantation. **Patients** for whom chimerism could not be assessed through STR-PCR such as cases who received allo-HSCT from a monozygotic (identical) twin, were excluded from the study.

Patients' average age was 11.57±6.83 years old (ranged 2 to 30 years old). Transplanted patients consisted of a wide variety of diseases such as acute leukemia, Fanconi's anemia, aplastic anemia, thalassemia major, immunodeficiency diseases (e.g. chronic granulomatosis,

histiocytosis X), and osteoporosis. Most of the patients were referred for bone marrow transplantation after the occurrence of first or second relapse of the disease. Of all investigated patients, 12 were children with acute leukemia who were analyzed separately. The EDTA- blood samples were collected from both recipients and donors before BMT. Moreover, bone marrow aspiration samples were taken from recipients on day 14 or 15 after BMT.

DNA was extracted by YTA kit (Genomic DNA Blood/culture cell mini-kit. Cat No: YT9040, IRAN). The method used for chimerism analysis in this study was STR-PCR using 7 STR indicators. The STR loci includedD\_3 S\_3045,D\_4 S\_2366 D\_12 S 1064·D 16 S 539, HUMTHO1,D 13 S 317, and F\_13 A 1. Relevant information regarding these indicators is presented in Table I. The possible relationships between the extent of chimerism and acute GVHD, chronic GVHD, disease relapse, and transplantation survival were analyzed in all patients including children with acute leukemia.

The STR-PCR reaction was prepared at a final volume of 27.5 µl using 10 µl DW, 12.5 µl Taq DNA Polymerase Master Mix RED (Amplicon Company, DENMARK), 4 µl mixed primers 10 pmol, and 1 µl DNA. PCR amplification was performed in a thermocycler (Applied Biosystems, USA) under the following cycling conditions: an initial denaturation at 94, °C for 4 min, followed by 7 amplification cycles of 95 °C for 40 sec, 62 °C for 40 sec, 72°C for 40 sec and 33 amplification cycles of 94 °C for 40 sec, 55 °C for 40 sec and 72 °C for 40 sec with an ultimate extension at 72 °C for 5 min. PCR separated products were 8% polyacrylamide gel electrophoresis and visualized with the UV light after ethidium All bromide staining. clinical data including relapse incidence, **GVHD** occurrence-its type and stage, and survival were extracted from patients' medical records.

## Statistical analysis

Statistical analysis was performed using SPSS (version 16). Clinical and laboratory characteristics were analyzed by descriptive statistics. Analysis of survival data was done using Kaplan-Meier method and log-rank test. The statistical level of significance was defined as p-value less than 0.05.

### **Results**

The study group consisted of 30 patients who received allogeneic hematopoietic stem cell transplantation (allo-HSCT) during 2012-2016 at Montaserie Hospital, Mashhad, Iran. Of these, 90% (27) individuals) were children and 10% (3 individuals) were adults. In the children group, 44.44% (12 patients) had acute leukemia, while 55.5% (15 patients) were diagnosed with other diseases. In children diagnosed with acute leukemia, 50% (6 individuals) had acute lymphoblastic leukemia, while 50% (6 individuals) had acute myeloblastic leukemia. conversion of chimerism occurred in one child due to receiving BMT from a monozygotic (identical) twin and hence was excluded from the study. In the adult group, 1 patient (33.33%) had acute leukemia, while 2 patients (66.67%) were other hematological diagnosed with diseases. The median age of the patients was 11.57±6.83 years old (ranged from 2 to 30 years old), and the median follow-up duration was 18.07±12.57 months (range 1-49 months). The average interval between transplantation and relapse was  $0.46\pm1.43$  months (range 0-5 months), while the average interval between transplantation and death was 2.31±5.58 months (range 0-23months). In addition, 73.3% (22)patients) of allo-HSCT recipients survived and 26.7% (8 patients) expired during the study period. Relapse was not observed in 90% of the total population, and averages of death and

relapse were reported 0.27 and 0.1, respectively.

Regarding chimerism, while 6.7% (2 patients) developed no chimerism, mixed chimerism (between 5%-95% cells of donor origin in hematopoietic tissue), and complete chimerism (more than 95% cells of donor origin) were observed in 13.3% (4 patients) and 76.7% (23 patients), respectively. All the data regarding death, relapse, and chimerism status of cases with acute leukemia are reported separately in Table II. The overall average percentage of chimerism was 84.66±26.45 (range 5-95). The average chimerism rates for survived and expired patients were 90±19.27 and 67.86±39.14, respectively. The average chimerism percentage in relapsed and nonrelapsed patients was 57.5±53.03 and 86.67±24.17, respectively. No significant association was found between the average percentage of chimerism and relapse (P = 0.13) (t = 1.54).

Regarding patients' survival rates, as shown in Figure 1, average survival was  $36.47\pm3.71$  (CI: 29.19 – 43.76). The average survival rates in non-relapsed patients, non-relapsed patients complete chimerism, and non-relapsed patients without complete chimerism were  $43.49\pm2.94$  (CI: 37.71-49.27),  $46.6\pm2.31$ , and 25±5.71, respectively (Figure 5). Considering non-relapsed patients with and without pediatric acute leukemia, the average survival rates were 24.21±2.93 (CI: 18.47 - 29.96) and 41.42±4.88 (CI: 31.84 – 51.00), respectively (Figure 3). Having compared average survival based on patients' initial disease, no significant difference among patients was detected  $(\chi 2= 0.32, P = 0.85)$ . Similarly, survival rates of children with acute leukemia showed no significant difference compared to those without acute leukemia ( $\chi 2 = 0.37$ ) (P= 0.8). While survival rates of patients with and without complete chimerism showed a significant difference ( $\chi 2 = 4.14$ ) (P= 0.04) (Figure 4), estimates of survival based on the percentage of chimerism showed no significant difference (P = 0.052, t = 2.03). Additionally, survival analysis in both patients with and without chimerism suggested complete significant association between these two groups and relapse incidence ( $\chi 2 = 2.31$ , P= 0.12). Patients' average survival with respect to the interval between transplantation and death considering their initial disease is also separately shown in Figure 2.

Concerning GVHD, there was no significant difference in incidence frequencies of different types of GVHD in patients with and without chimerism (P value: 0.82) (Table 2 and Table III). Additionally, there was no significant difference regarding frequency of skin GVHD among children with acute leukemia and their level of chimerism (P value: 0.51). As shown in Table IV, regarding the association between the average chimerism level and different types of GVHD, only significant association was found between skin GVHD and average chimerism (P= 0.02). All data regarding skin, liver, gastrointestinal, and chronic GVHD are also illustrated in Table II.

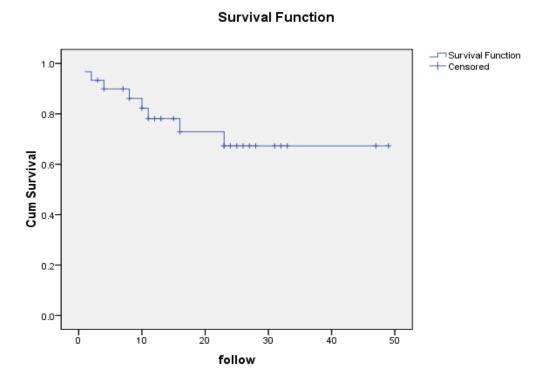


Figure 1: Patients' survival rate based on the follow-up time

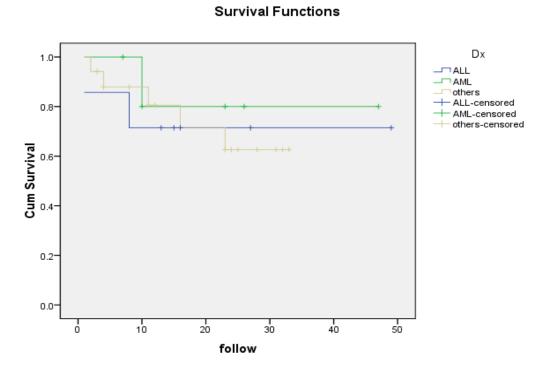


Figure 2: Patients' survival based on their death intervals (in month) and initial disease

# **Survival Functions** kaimetotal \_\_no yes no-censored yes-censored Cum Survival 0.2 0.0 10 20 30 40 ó 50 follow

Figure 3: Patients survival based on the presence or absence of pediatric acute leukemia

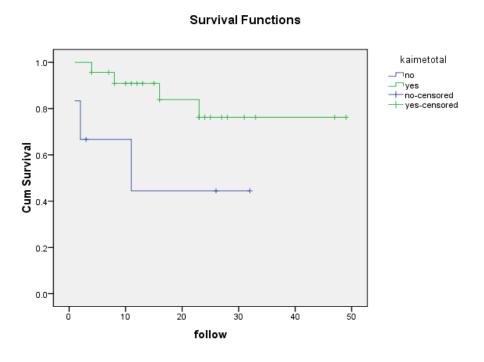


Figure 4: Survival rate based on presence or absence of complete chimerism

# **Survival Function**

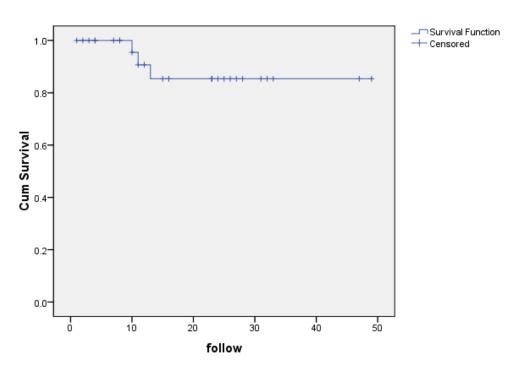


Figure 5: Survival rate in patients without relapse

Table I: Different STR indicators used in the present study

	Locus	Marker	Primer sequence		
	$D_3S_{3045}$	GATA84B12	F: ACCAAATGAGACAGTGGCAT		
			R: ATGAGGACGGTTGACATCTG		
	D <sub>4</sub> S <sub>2366</sub>	GATA22G05	F: TCCTGACATTCCTAGGGTGA		
			R:AAAACAAATATGGCTCTATCTATCG		
	$D_{12}S_{1064}$	GATA63D12	F: ACTCCAAGGTTCCAGCC		
			R:AATATTGACTTTCTCTTGCTACCC		
	D <sub>16</sub> S <sub>539</sub> GATA11C06		F:GATCCCAAGCTCTTCCTCTT		
			R:ACGTTTGTGTGTGCATCTGT		
	HUMTHO1	AATG	F:GTGGGCTGAAAAGCTCCCGATTAT		
			R:ATTCAAAGGGTATCTGGGCTCTGG		
	$D_{13}S_{317}$	GATA7G10	F:ACAGAAGTCTGGGATGTGGA		
			R:GCCCAAAAAGACAGACAGAA		
	$F_{13}A_{1}$	n/a	F:GAGGTTGCACTCCAGCCTTT		
			R:ATGCCATGCAGATTAGAAA		

[ Downloaded from ijpho.ssu.ac.ir on 2025-11-04 ]

Table II: Distribution of patients in terms of initial disease, the incidence of chronic GVHD, relapse, death, different types of GVHD, and chimerism status

Variable		Numbers (%)	ALL	AML	Chimerism Status			
					Complete	No	All	
						Complete		
Death	No	22(73.3%)	5 (71.4%)	5 (83.3%)	19 (82.6%)	2 (50%)	21 (77.8%)	
	Yes	8 (26.7%)	2 (28.6%)	1 (16.7%)	4 (17.4%)	2 (50%)	6 (22.2%)	
Relapse	No	27 (90%)	6(85.7%)	5 (83.3%)	22 (95.6%)	3 (75%)	25 (25%)	
	Yes	3 (10%)	1(14.3%)	1 (16.7%)	1 (4.3%)	1 (25%)	2 (7.4%)	
Chronic	No	22 (73.3%)	5	4	16 (69.6%)	3 (75%)	19 (70.4%)	
GVHD			(71.4%)	(66.7%)				
	Yes	8 (26.7%)	2	2	7 (30.4%)	1 (25%)	8 (29.6%)	
			(28.6%)	(33.3%)				
Skin GVHD	No	11 (36.7%)	4 (57.1%)	2	7 (30.4%)	1 (25%)	8 (29.6%)	
				(33.3%)				
	Yes	19 (63.3%)	3	4	16 (69.6%)	3 (75%)	19 (70.4%)	
			(42.9%)	(66.7%)				
Liver GVHD	No	22 (73.3%)	7	2	16 (69.6%)	3 (75%)	19 (70.4%)	
			(100%)	(33.3%)				
	Yes	8 (26.7%)	0	4	7 (30.4%)	1 (25%)	8 (29.6%)	
			(0%)	(66.7%)				
Gastrointestin	No	18 (60%)	3	4	13 (56.4%)	2 (50%)	15 (66.6%)	
al GVHD			(42.9%)	(66.7%)				
	Yes	12 (40%)	4	2	10 (43.6%)	2 (50%)	12 (43.4%)	
			(57.1%)	(33.3%)				

Table III: Frequency of different types of GVHD concerning chimerism status

		Levels	Frequency of	ALL	AML	Other	Chimerism Status		
			GHVD level	N (%)	N (%)		Complete	No <b>Complete</b>	All
	Chronic GVHD								
	Skin GVHD	0	11 (36.5%)	4 (57.1%)	2 (33.3%)	5 (29.4%)	7 (30.4%)	3 (50%)	10 (34.5%)
		1	4 (13.3%)	3 (42.9%)	0 (0%)	1 (5.9%)	3 (13%)	1 (16.7%)	4 (13.8%)
		2	2 (6.7%)	0 (0%)	0 (0%)	2 (11.8%)	2 (8.7%)	0 (0%)	(6.9%)
		3	6 (20%)	0 (0%)	1 (16.7%)	5 (29.4%)	5 (21.7)	1 (16.7%)	6 (20.7%)
		4	7 (23.3%)	0 (0%)	3 (50%)	4 (23.5%)	6 (26.1)	1 (16.7%)	7 (24.1%)
	Liver GVHD	0	22 (73.3%)	7 (100%)	2 (33.3%)	13	16 (69.6%)	5 (83.3%)	21 (72.4%)
		1	1 (3.3%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)	1 (16.7%)	1(3.4%)
		2	5 (16.7%)	0 (0%)	3 (50%)	2 (11.8%)	5 (21.7%)	0 (0%)	5 (17.2%)
		3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		4	2 (6.7%)	0 (0%)	0 (0%)	2 (11.8%)	2 (8.7%)	0 (0%)	2 (6.9%)
	Gastrointestinal GVHD	0	18 (60%)	3 (42.9%)	4 (66.7%)	11 (64.7%)	13 (56.5%)	4 (66.7%)	17 (58.6%)
		1	1 (3.3%)	1 (14.3%)	0 (0%)	0 (0%)	1 (4.3%)	0 (0%)	(3.4%)
11-04		2	6 (20%)	2 (28.6%)	1 (16.7%)	3 (17.6%)	5 (21.7%)	1 (16.7%)	6 (20.7%)
ir on 2025		3	2 (6.7%)	1 (14.3%)	1 (16.7%)	0 (0%)	1 (4.3%)	1 (16.7%)	(6.9%)
ijpho.ssu.ac.ir on 2025-11-04]		4	3 (10%)	0 (0%)	0 (0%)	3 (17.6%)	3 (13%)	0 (0%)	3 (10.3%)

Table IV: Association between GVHD status and average chimerism

GVHD status	Percentage of average chimerism	P-value
patients with skin GVHD	92.63±6.53	( P= 0.02)
patients without skin GVHD	69.5±41.26	
patients with digestive GVHD	93.33±4.43	(P= 0.14)
patients without digestive GVHD	78.53±33.39	
patients with liver GVHD	80.95±30.43	( P= 0.22)
patients without liver GVHD	94.38±1.76	
patients with chronic GVHD	80.95±30.43	(P= 0.22)
patients without chronic GVHD	94.38±1.76	
patients with transplantation versus	88.86±19.69	(P = 0.13)
patients without transplantation versus	71.43±40.48	
Patient with acute leukemia	85.5±28.32	(P = 0.756)
Patient without acute leukemia		

### Discussion

Allogenic bone marrow transplantation is currently identified as a curative therapy for both hematological malignancies and non-neoplastic genetic disorders (19). Monitoring of chimerism is regarded as a useful diagnostic tool to evaluate the risk of relapse and GVHD after allogenic bone further marrow transplantation, and improvements of chimerism analysis methods may result in better allogenic bone marrow transplantation outcomes (20). In the present study, bone marrow samples were used to analyze chimerism and its association with transplantation suggested outcome. Some studies performing early chimerism assessment on day 3 to 7 after allogenic transplantation. However, other researchers recommended chimerism analysis on day 1 to 14 after transplantation. In the current study, chimerism analysis was carried out on day

14 or 15 after BMT (16, 21). In routine practice, early analysis chimerism is considered as a prognostic predictive indicator of outcomes. However, some studies showed that frequent assessment of chimerism at close intervals could be a better approach. Liesveld et al., recommended a weekly analysis of chimerism by 100 days after BMT (16). In the present study, the association between chimerism analysis and factors such as relapse, survival, and GVHD in both adult and children and relation with their background disease were assessed. Besides, the association between these factors and pediatric acute leukemia was investigated. Until recently, this association between mixed chimerism leukemia and non-leukemia transplanted patients has been studied. However, results regarding this issue remain controversial and further

investigations are needed (1, 4). previous studies proposed significant relationship between relapse incidence and mixed chimerism, other investigators found no significant association. In a study conducted by Lee et al., donor-specific T cell chimerism was evaluated in patients with acute myeloblastic leukemia and myelodysplastic syndrome (MDS). They suggested chimerism 80% > as an indicator of BMT failure in patients transplanted after first or second relapse. However, this association was not found in patients transplanted after third or more relapse occurrences. Moreover, Ghafari et al., found no significant association between relapse and complete or mixed chimerism (1). Similarly, Barrios et al., reported lack of association between mixed chimerism and relapse in children (22). In the current study, no significant association was found between relapse and complete chimerism (P = 0.12). Besides, no significant relationship was found when comparing relapse rates in patients with complete chimerism and those with mixed chimerism (P = 0.15). Average chimerism percentage in relapsed patients reported to be less than non-relapsed patients in previous studies. However, in this study, the difference was not statistically significant (P = 0.13). survival, Regarding no significant association was found between total transplanted population and children with acute leukemia or other subjects with acute In contrast, there was leukemia. significant association between complete chimerism and patients' survival compared to patients with mixed chimerism or without chimerism. (P = 0.04). The current study found that many transplanted patients with mixed chimerism developed no graft rejection or severe signs of GVHD. Similarly, some previous studies found no relationship between GVHD and chimerism. For instance, Pegs et al., reported no association between GVHD and mixed chimerism (24). In a study by

Ghafari et al., the incidence rate of acute GVHD in patients with mixed chimerism was significantly less than those with complete chimerism (P= 0.1). However, no association was reported between severity and levels of acute and chronic GVHD with chimerism grade (1).

Ballon et al., showed a significant correlation between the incidence time of complete hematopoietic chimerism and GVHD emergence (25). In the present study, no association was found between acute GVHD including skin, liver, and gastrointestinal tract GVHD and stage of complete or mixed chimerism in both total population and children with acute leukemia, However, there was a significant association between acute skin GVHD and average chimerism level of patients (P = 0.002). Regarding the association between relapse and chimerism level, different results of the present study compared to previous studies can be attributed to the small number of patients with posttransplantation relapse or use of a different definition of mixed chimerism which was < 80 in some studies, while in the current mixed chimerism study. the considered  $\leq$  95 (9). Another reason for this inconsistency can be lie in different time and number of chimerism analysis. Various studies suggested multiple chimerism assessment after transplantation as a model that can give more precise information for the prediction of relapse and GVHD and subsequent treatment interventions (1, 4, 16). It seems that donor-specific T cell chimerism analysis can provide more precise data regarding the incidence of relapse and GVHD. Lineage-specific cell chimerism should be considered as a selective approach for adjusting non-myeloablative treatment and reducing the severity of the medication regime. In the current study, the chimerism analysis was not limited to a lineagespecific cell type but it assessed the percentage of different types of donorderived hematopoietic cells. Frequent assessments of chimerism status during the

first 100 days following BMT and less frequent analysis after 100 days should be implemented in clinical practice for the prediction of relapse and GVHD. In addition, early-stage chimerism patterns after transplantation can be regarded as a predictive indicator of acute GVHD such as skin GVHD in the present study and it can be also used for early treatment interventions.

### Conclusion

Chimerism analysis after allo-HSCT is an important tool in the assessment of graft status and function. Chimerism analysis can be helpful for the prediction of posttransplant adverse effects such as GVHD graft rejection. Furthermore, and chimerism assessment can assist physicians to choose the best possible treatment interventions. It is worth mentioning that frequent chimerism assessments at close intervals may provide precise prediction more transplantation outcome. Moreover. donor-specific evaluation of T chimerism has been suggested as a more specific test that helps physicians to adjust therapeutic regimens after transplantation.

### **Conflict of interest**

Authors declared no conflict of interest.

### References

- 1. Ghaffari H, Alimoghaddam K, Foroughi F, Chahardouli B, Sanaat Z, Bahar B et al. Effects of chimerism on graft-versus-host disease, disease recurrence and survival after HLA-identical marrow transplantation in Iran. Blood J 2005; 5(2): 431-438.
- 2. Raina A, Chaudhary G, Dogra TD, Khandelwal D, Balayan A, Jain V, et al. Benefit of STR-based chimerism analysis to identify TA-GVHD as a cause of death: Utility of various biological specimens. Med Sci Law 2016;56(2):142-146.
- 3. Mousavi SA, Javadimoghadam M, Ghavamzadeh A, Alimoghaddam K,

- Sayarifard A, Ghaffari AH, et al. The Relationship between STR-PCR Chimerism Analysis and Chronic GvHD Following Hematopoietic Stem Cell Transplantation. Int J Hematol Oncol Stem Cell Res 2017; 11(1): 24–29.
- 4. Khan F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. Bone marrow transplant 2004;34(1):1-12
- 5. Preuner S, Peters C, Pötschger U, Daxberger H, Fritsch G, Geyeregger R, et al. Risk assessment of relapse by lineage-specific monitoring of chimerism in children undergoing allogeneic stem cell transplantation for acute lymphoblastic leukemia.

  Haematologica 2016;101(6):741-746.
- 6. Chen CT, Gau JP, Liu JH, Chiou TJ, Hsiao LT, Liu YC. Early achievement of full donor chimerism after allogeneic hematopoietic stem cell transplantation predicts lower relapse risk in patients with acute lymphoblastic leukemia. J Chin Med Assoc 2018;81(12):1038-1043.
- Leiman M. Zaucha-Prażmo A. Zawitkowska J, Mroczkowska A, Grabowski D, Kowalczyk JR, et al. Impact of early chimerism status on clinical outcome in children with acute lymphoblastic leukaemia after haematopoietic stem cell transplantation. BMC Cancer 2019;19(1):1141-1149.
- 8. Haugaard AK, Madsen HO, Marquart HV, Rosthøj S, Masmas TN, Heilmann C, et al
- Highly sensitive chimerism detection in blood is associated with increased risk of relapse after allogeneic hematopoietic cell transplantation in childhood leukemia.Pediatr Transplant 2019;23(7):e13549-e1355.
- 9. Lee HC, Saliba RM, Rondon G, Chen J, Charafeddine Y, Medeiros LJ, et al. Mixed T lymphocyte chimerism after allogeneic hematopoietic transplantation is predictive for relapse of acute myeloid leukemia and myelodysplastic syndromes.

- Biol Blood Marrow Transplant 2015; 21(11):1948-1954.
- 10. Garnett C, Apperley JF, Pavlů J. Treatment and management of graft-versus-host disease: improving response and survival. Ther Adv Hematol 2013;4(6):366-378.
- 11. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis 2007; 2(1):35-42.
- 12. Sachs DH, Kawai T, Sykes M. Induction of tolerance through mixed chimerism. Cold Spring Harb Perspect Med 2014;4(1):a015529-a015533.
- 13. Andreani M, Testi M, Lucarelli G. Mixed chimerism in haemoglobinopathies: from risk of graft rejection to immune tolerance. Tissue Antigens 2014;83(3):137-146.
- 14. Cetrulo CL Jr, Drijkoningen T, Sachs DH. Tolerance induction via mixed chimerism in vascularized composite allotransplantation: is it time for clinical application? Curr Opin Organ Transplant 2015; 20(6):602-607.
- 15. Lui KO, Howie D, Ng SW, Liu S, Chien KR, Waldmann H. Tolerance induction to human stem cell transplants with extension to their differentiated progeny. Nat Commun 2014; 5:5629-5633.
- 16. Liesveld J, Rothberg P. Mixed chimerism in SCT: conflict or peaceful coexistence? Bone marrow transplant 2008;42(5):297-310.
- 17. Lion T, Watzinger F, Preuner S, Kreyenberg H, Tilanus M, de Weger R, et al. The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation. Leukemia 2012: 26(8):1821-1828.
- 18. Clark JR, Scott SD, Jack AL, Lee H, Mason J, Carter GI, 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. Br J Haematol 2015;168(1):26-37. 19. Aljurf M, Abalkhail H, Alseraihy A, Mohamed SY, Ayas M, Alsharif F, 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. Biotechnol Res Int 2016; 20(16):8589270-858975.
- 20. Gineikiene E, Stoskus M, Griskevicius L. Recent advances in quantitative chimerism analysis. Expert Rev Mol Diagn 2009; 9(8):817-832
- 21. Lion T, Daxberger H, Dubovsky J, Filipcik P, Fritsch G, Printz D, et al. Analysis of chimerism within specific leukocyte subsets for detection of residual or recurrent leukemia in pediatric patients after allogeneic stem cell transplantation. Leukemia 2001;15(2):307-310.
- 22. Barrios M, Jiménez-Velasco A, Román-Gómez J, Madrigal ME, Castillejo JA, Torres A, et al. Chimerism status is a useful predictor of relapse after allogeneic stem cell transplantation for acute leukemia. Haematologica 2003; 88(7):801-810.
- 23. Walters MC, Patience M, Leisenring W, Rogers ZR, Aquino VM, Buchanan GR, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 2001;7(12):665-673.
- 24. Peggs KS, Thomson K, Hart DP, Geary J, Morris EC, Yong K, et al. Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. Blood 2004;103(4):1548-1556.
- 25. Balon J, Hałaburda K, Bieniaszewska M, Reichert M, Bieniaszewski L, Piekarska A, et al. Early complete donor hematopoietic chimerism in peripheral blood indicates the risk of extensive graftversus-host disease. Bone marrow transplant 2005; 35(11):1083-1088.