Original Article *Iran J Ped Hematol Oncol. 2020, Vol 10, No 2, 74-86*

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

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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 monitoring after BMT can provide valuable information that enables clinicians to early detect disease relapse and GVHD and facilitates further therapeutic interventions. The term chimerism refers to the presence of hematopoietic cells of donor origin after bone marrow transplantation. The 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 lympho-hematopoietic 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 (1, 4). Chimerism analysis takes advantage of polymorphic short tandem repeat markers within the genome to discriminate donor from recipient DNA (2-4). The 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 during 2012-2016. This study was 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 Qaem Hospital, Mashhad, Iran, on day 14 or 15 after BMT. All included patients were qualified for allogenic hematopoietic 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 post-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 products were separated by 8% polyacrylamide gel electrophoresis and visualized with the UV light after ethidium bromide staining. All 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. No 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 diagnosed with other hematological 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±1.43 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 non-relapsed 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±3.71 (CI: 29.19 – 43.76). The average survival rates in non-relapsed patients, non-relapsed patients with complete chimerism, and non-relapsed patients without complete chimerism were 43.49±2.94 (CI: 37.71 – 49.27), 46.6±2.31, 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 (Figure3). Having compared average survival based on patients' initial disease, no significant difference among patients was detected ($\chi2$= 0.32, P = 0.85). Similarly, survival rates of children with acute leukemia showed no significant difference compared to those without acute leukemia ($\chi2$= 0.37) (P= 0.8). While survival rates of patients with and without complete chimerism showed a significant difference ($\chi2$= 4.14) (P= 0.04) (Figure4), 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 complete chimerism suggested no significant association between these two groups and relapse incidence ($\chi2$= 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 complete chimerism (P value: 0.82) (Table 2 and Table III). Additionally, there was no significant difference regarding the 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 a 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.

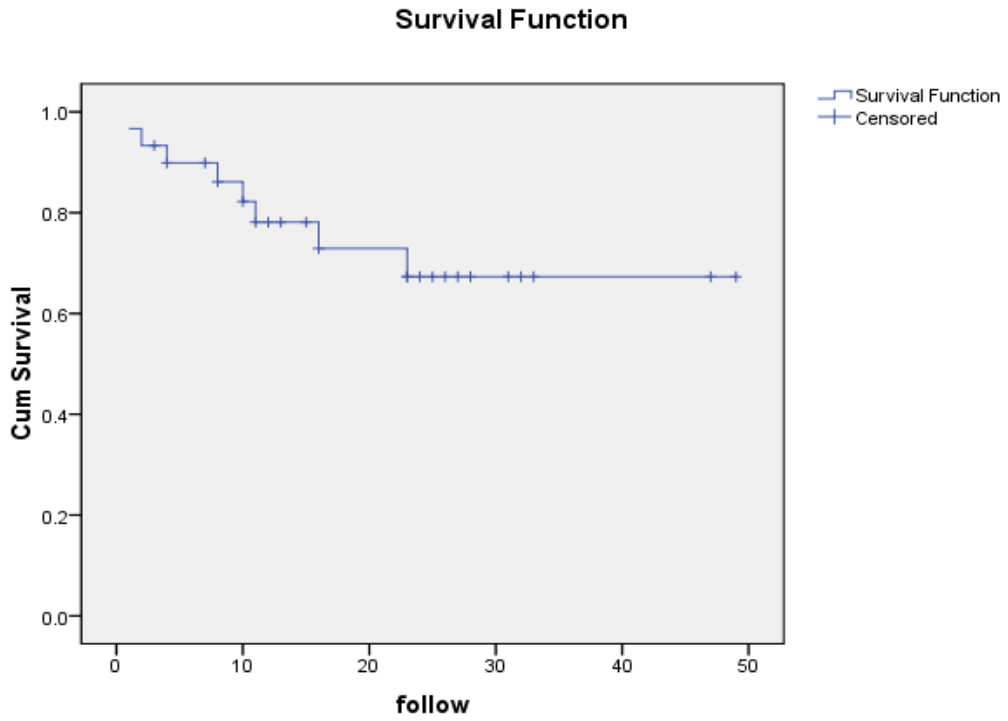

Figure 1: Patients' survival rate based on the follow-up time

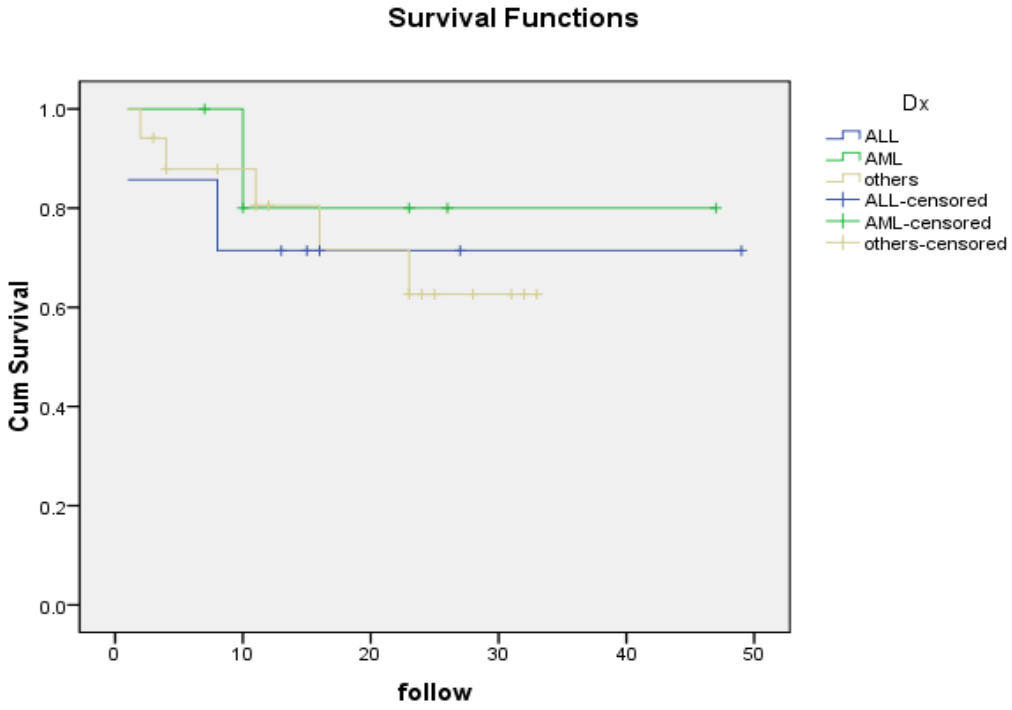

Figure 2: Patients' survival based on their death intervals (in month) and initial disease

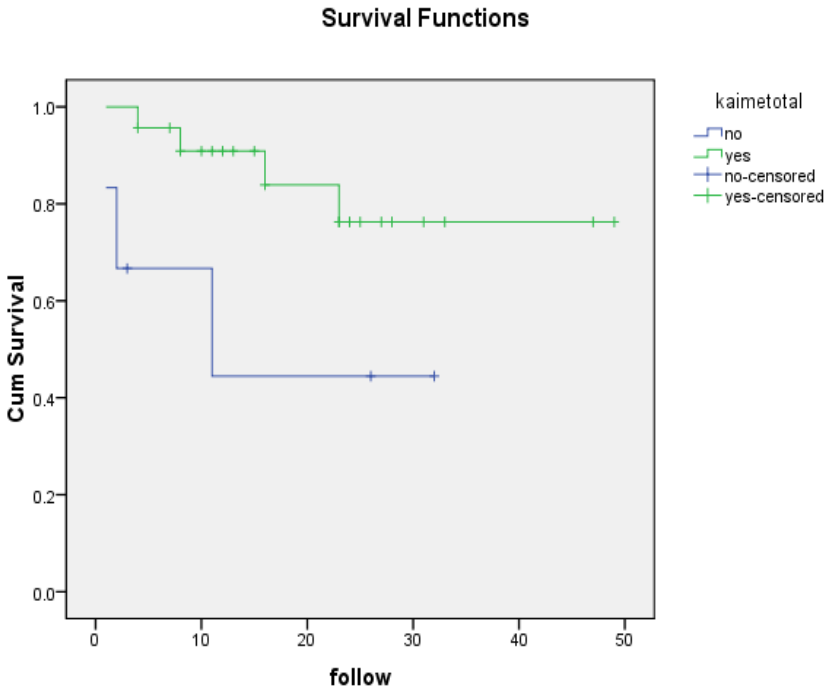

Figure 3: Patients survival based on the presence or absence of pediatric acute leukemia

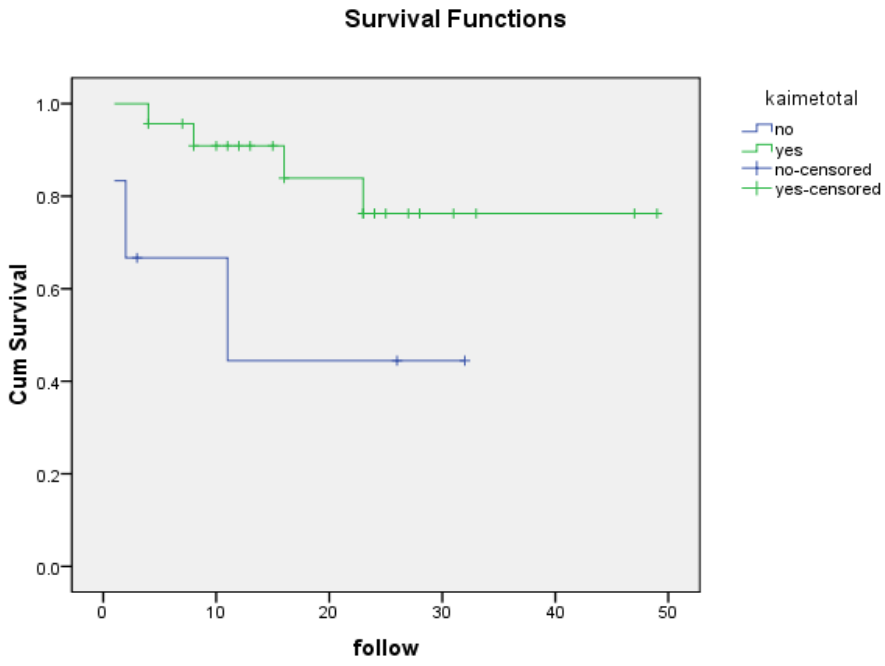

Figure 4: Survival rate based on presence or absence of complete chimerism

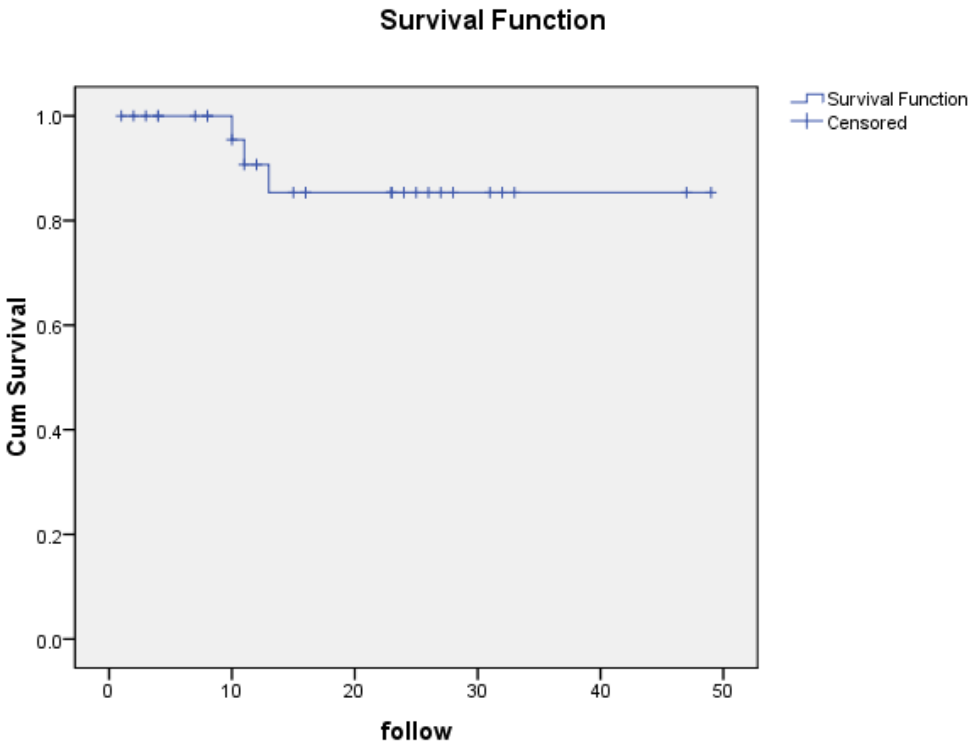

Figure 5: Survival rate in patients without relapse

Table I: Different STR indicators used in the present study

	Locus	Marker	Primer sequence
	D_3S_{3045}	GATA84B12	**F: ACCAAATGAGACAGTGGCAT**
			R: ATGAGGACGGTTGACATCTG
	D_4S_{2366}	GATA22G05	**F: TCCTGACATTCCTAGGGTGA**
			R:AAAACAAATATGGCTCTATCTATCG
	$D_{12}S_{1064}$	GATA63D12	**F: ACTCCAAGGTTCCAGCC**
			R:AATATTGACTTTCTCTTGCTACCC
	$D_{16}S_{539}$	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

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 Complete	All
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 GVHD	**No**	22 (73.3%)	5 (71.4%)	4 (66.7%)	16 (69.6%)	3 (75%)	19 (70.4%)
	Yes	8 (26.7%)	2 (28.6%)	2 (33.3%)	7 (30.4%)	1 (25%)	8 (29.6%)
Skin GVHD	**No**	11 (36.7%)	4 (57.1%)	2 (33.3%)	7 (30.4%)	1 (25%)	8 (29.6%)
	Yes	19 (63.3%)	3 (42.9%)	4 (66.7%)	16 (69.6%)	3 (75%)	19 (70.4%)
Liver GVHD	**No**	22 (73.3%)	7 (100%)	2 (33.3%)	16 (69.6%)	3 (75%)	19 (70.4%)
	Yes	8 (26.7%)	0 (0%)	4 (66.7%)	7 (30.4%)	1 (25%)	8 (29.6%)
Gastrointestinal GVHD	**No**	18 (60%)	3 (42.9%)	4 (66.7%)	13 (56.4%)	2 (50%)	15 (66.6%)
	Yes	12 (40%)	4 (57.1%)	2 (33.3%)	10 (43.6%)	2 (50%)	12 (43.4%)

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

	Levels	Frequency of GHVD level	ALL N (%)	AML N (%)	Other	Chimerism Status		
						Complete	No **Complete**	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%)	2 (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%)	1 (3.4%)
	2	6 (20%)	2 (28.6%)	1 (16.7%)	3 (17.6%)	5 (21.7%)	1 (16.7%)	6 (20.7%)
	3	2 (6.7%)	1 (14.3%)	1 (16.7%)	0 (0%)	1 (4.3%)	1 (16.7%)	2 (6.9%)
	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 marrow transplantation, and further 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 outcome. Some studies suggested 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 clinical practice, early analysis of chimerism is considered as a prognostic and predictive indicator of BMT 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 and leukemia and non-leukemia in transplanted patients has been studied. However, results regarding this issue remain controversial and further

investigations are needed (1, 4). While some previous studies proposed a 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 was reported to be less than non-relapsed patients in previous studies. However, in this study, the difference was not statistically significant (P = 0.13). Regarding survival, no significant association was found between total transplanted population and children with acute leukemia or other subjects with acute leukemia. In contrast, there was a 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 post-transplantation relapse or use of a different definition of mixed chimerism which was ≤ 80 in some studies, while in the current study, the mixed chimerism was considered ≤ 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 lineage-specific cell type but it assessed the percentage of different types of donor-derived 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 post-transplant adverse effects such as GVHD and graft rejection. Furthermore, 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 a more precise prediction of transplantation outcome. Moreover, evaluation of donor-specific T cell 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.

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