

Mixed Donor Chimerism in Non-Malignant Haematological Diseases after Allogeneic Bone Marrow Transplantation

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ABSTRACT

Objective: To determine the frequency of mixed donor chimerism in patients of non-malignant haematological diseases after allogeneic bone marrow transplant.

Study Design: A cross-sectional, observational study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from July 2010 to June 2011.

Methodology: Donor chimerism was assessed in patients of aplastic anaemia and beta-thalassaemia major who underwent allogeneic bone marrow transplantation (BMT). Peripheral blood samples were used to assess chimerism status by analysis of short tandem repeats (STR). In patients where pre-transplant blood sample was not available, swab of buccal mucosa was used for pre-transplant STR profile. A standard set of primers for STR markers were used and the amplified DNA was resolved by gel electrophoresis and stained with silver nitrate. The percentage of donor origin DNA was estimated by densitometer.

Results: Out of 84 patients, 52 (62%) were males, while 32 (38%) were females. In patients of beta-thalassaemia major, 31 (62%) developed mixed donor chimerism (MC), 13 (26%) developed complete donor chimerism (CC) and 6 (12%) had graft failure. In aplastic anaemia, 17 patients (50%) achieved MC, 13 (38.2%) had CC and 4 (11.8%) developed graft failure. The combined frequency of mixed donor chimerism for both the diseases was 58.3%. D3S1358 was the most informative STR marker in these patients.

Conclusion: Majority of the studied patients developed mixed donor chimerism following bone marrow transplantation, whereas only a minor percentage of the patients had graft failure. Analysis of D3S1358 was the most informative in assessing donor chimerism in patients who underwent BMT.

Key words: *Mixed donor chimerism. Allogeneic bone marrow transplantation. Short tandem repeats.*

INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is widely used as curative treatment for patients suffering from malignant as well as non-malignant haematological diseases.¹ It can be accomplished by either bone marrow transplantation (BMT), or peripheral blood stem cell transplantation or cord blood stem cell infusion.² Aplastic anaemia, beta-thalassaemia major, chronic myeloid leukemia and acute leukemia form the bulk of diseases for which BMT is carried out in Pakistan.³

Following a BMT, the recipient develops varying degrees of chimerism of the donor and recipient cells.⁴ This chimerism can be broadly categorized as complete or mixed depending upon the percentage of donor cells in the recipient's blood or bone marrow.⁵ Monitoring the chimerism status is valuable in determining the success of allogeneic stem cell transplant⁶⁻⁹ and also following the course of mixed donor chimerism patients.

Chimerism testing involves identifying the genetic profiles of the recipient and of the donor and then evaluating the extent of mixture in the recipient's blood or bone marrow.^{4,5}

Analysis of the genetic profile by short tandem repeats (STR) using polymerase chain reaction (PCR) is the preferred method for assessing chimerism.¹⁰⁻¹² STRs are highly polymorphic regions of extragenic DNA that consists of a sequence of 2 to 8 bases in length.¹³ The sequence is repeated in tandem i.e. one after another like carriages of a train and it is the number of these repeats that vary from individual to individual. The amplified STR markers can either be resolved on capillary electrophoresis by a genetic analyzer,¹⁴ or by gel electrophoresis which can later on be quantified. A pre-transplant genetic profile of the recipient done by STR analysis should be available ideally for comparison with the post-transplant STR profile. However, if it is not available, then a sample of the buccal mucosa of the recipient taken carefully with a swab can be used for extraction of the DNA and it can be treated as pre-transplant STR genetic profile.

Despite its immense potential and utility, studies pertaining to donor chimerism have not been done in Pakistan. Therefore, information regarding this important

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Received May 07, 2012; accepted September 11, 2012.

parameter in post-BMT patients is deficient. This study describes in detail the materials required and the procedure followed for chimerism analysis best suited for our setup. It also highlights the STR markers which are most informative.

The aim of this study was to determine the frequency of mixed donor chimerism in patients of non-malignant haematological diseases after allogeneic bone marrow transplant.

METHODOLOGY

It was cross-sectional study and was carried out in the Department of Haematology at Armed Forces Institute of Pathology, Rawalpindi. A total of 84 patients; 50 with beta-thalassaemia major and 34 with aplastic anaemia were part of the study. Patients of either gender who had a post-transplant duration between 2 months to 2 years were included in the study, whereas patients who were undergoing a second transplant after a graft failure were excluded. Sample size was calculated by WHO sample size calculator; using 95% confidence interval and expected frequency of mixed chimerism in 32.2%,¹⁵ and absolute precision as 10%.

Mixed donor chimerism was defined as a percentage of donor origin DNA between 5 – 95% in the recipient's blood or bone marrow as assessed by STR-PCR analysis. It was further sub-divided into two categories of > 50% mixed donor chimerism and < 50% mixed donor chimerism. Patients with > 95% donor DNA in blood or bone marrow were labeled as complete chimerism (CC), while those with < 5% were regarded as graft failure.

Aseptic technique was used to sample 2.5 – 3 ml venous blood in ethylene diamine tetra-acetic acid (EDTA) and complete blood counts were performed on Sysmex KX - 21 to assess the total leukocyte count (TLC). In individuals with low TLC; bone marrow aspirate collected in EDTA was used as a source of DNA. Most of the requests for donor chimerism analysis were post-transplant patients and in order to determine the patient's pre-transplant STR genotype, sample of the buccal swab was used to extract DNA.

DNA extraction was done using a commercial kit (Genra, USA); whereas amplification of the extracted DNA for STR markers was done by PCR. The contents included 2 uL of extracted DNA, 1 uL of the specific STR primer (Gene Link Company), 20 uL PCR mix and 0.1 uL Taq DNA polymerase. The detail of STR primers that were used is shown in Table I. Initial denaturation was done for 5 minutes at 94°C followed by 30 cycles of amplification with denaturation at 93°C for 48 seconds, annealing at 60°C for 48 seconds and extension at 72°C for one minute. The PCR amplified products of STR genetic profile/alleles were then resolved by electrophoresis on 8% polyacrylamide gel. The gel was stained with silver nitrate. Quantitative analysis of STR

alleles was done by densitometry. The selection of a particular STR marker in a patient was based on its informativeness in that patient.¹²

Results of the donor chimerism testing were analyzed using Statistical Package for Social Sciences (SPSS) version 13. Mean values and standard deviation were calculated for numerical data like age and duration of transplant. Frequencies of mixed donor chimerism, STR markers and gender of the patient were calculated.

RESULTS

Out of the total 84 patients, 52 (62%) were males and 32 (38%) females. Among the 50 patients of beta-thalassaemia major, 20 (40%) were females and 30 (60%) were males; while in aplastic anaemia, 22 (64.7%) were males and 12 (35%) were females. Collectively, the age ranged from 2 to 48 years. The overall mean age of the patients in the study was 10.3 ± 8.72 years; the mean age of beta-thalassaemia patients was 5.6 years and that of aplastic anaemia was 17.3 years. The source of stem cells in all the patients was a sibling.

The mean time of chimerism testing in aplastic anaemia patients was 9.5 ± 7.9 months; while that of beta-thalassaemia major patients was 8.3 ± 5.9 months. The most commonly used STR markers were for the loci,

Table I: The STR alleles that were tested and the chromosome on which they are located.

STR Locus	Primer sequence	STR Alleles (repeats)
D3S1358	F 5'-ACT GCA GTC CAA TCT GGG T R 5'-ATG AAA TCA ACA GAG GCT TG	14,15,16,17,18
FGA	F 5'-GCCCCATAGGTTTTGAACTCA R 5'-TGATTTGTCTGTAATTGCCAGC	19,21,22,23,24
D5S818	F 5'-GGGTGATTTCTCTTTGGT R 5'-TGATTCCAATCATAGCCACA	10,11,12,13
D7S820	F 5'-GTCATAGTTTGAACGAACTAACG R 5'-CTGAGGTATCAAAAAGCTCAGAGG	7,8,9,10,11
D8S1179	F 5'-TTTTTGTATTTTCATGTGTACATTCG R 5'-CGTAGCTATAATTAGTTTCATTTCA	10,11,12,13,14,15
TH01	F 5'-GTGGGCTGAAAAGCTCCCGATTAT R 5'-ATTCAAAGGGTATCTGGGCTCTGG	6,7,9,10
D21S11	F 5'-ATGATGAATGCATAGATGGATG R 5'-AATGTGTGCTTCCAGGC	27,28,29,30,31, 31.2,32.2,33.2, 35.2

Table II: Results of the short tandem repeat (STR) markers. The STR markers used versus the number of patients in which they were informative.

STR marker	Informativeness in number of patients	Percentage (%)
D3S1358	25	29.8
D5S818	23	27.4
D8S1170	18	21.4
D7S820	11	13.1
FGA	3	3.6
TH01	3	3.6
D21S11	1	1.2

Table III: Disease wise chimerism status of the patients in the study.

Disease	CC*	Mixed donor chimerism			Graft failure
		Total	> 50% MC	< 50% MC	
Aplastic anaemia	n = 13 (38.2%)	n = 17 (50.0%)	n = 13 (76.4%)	n = 4 (23.5%)	n = 4 (11.8%)
Beta-thalassaemia major	n = 13 (26%)	n = 31 (62%)	n = 22 (71.0%)	n = 9 (29.0%)	n = 6 (12.0%)

* Complete chimerism

D3S1358, D5S818 and D8S1170 (Table II). A total of 49 (58.3%) patients developed MC according to the criteria defined; while 25 (29.8%) patients achieved CC and 10 (11.9%) patients had graft failure.

Out of those who achieved MC, 35 (72.9%) patients had > 50% MC, while 13 (27.1%) patients had < 50% MC. The disease wise frequency of MC, CC and graft failure is shown in detail in Table III. The percentage of donor DNA present in the recipient hold prognostic significance; so it was also assessed in the patients that showed MC in the study. Among the patients of aplastic anaemia with > 50% MC, the mean quantity of donor cells was $86.3 \pm 9.15\%$ while among patients of beta-thalassaemia major with > 50% MC, it was $90.9 \pm 3.18\%$.

DISCUSSION

Evaluating the donor chimerism status in the recipient is crucial for assessing and monitoring engraftment of the transplanted stem cells in the recipient. Mixed donor chimerism occurs when both the donor and recipient origin DNA are detected in the recipient's blood or bone marrow. MC is an ongoing dynamic relation between the donor and recipient's cells and can be further sub-divided into stable, increasing, decreasing, or variably fluctuating (also called progressive mixed chimerism).^{5,16} Patients who achieve stable MC; irrespective of the percentage of donor DNA have excellent survival and no chronic graft versus host disease. Whereas recipients with progressive mixed chimerism have a higher incidence of developing graft rejection.

In the study, donor chimerism was determined using STR analysis, a PCR based technique and the results were resolved on gel electrophoresis. The percentage of donor DNA was measured on densitometer. Although the STR analysis was initially developed for use in forensic criminal identification,¹³ these markers can be effectively used to distinguish donor DNA from that of the recipient in an post BMT patient.^{17,18} Results of the study show that D3S1358, D5S818, D8S1170 were the three most frequently used STR markers for assessing chimerism in the study; used in 25, 23 and 18 patients respectively, i.e. a total of 66 patients (78.5%). The utility of these statistics is that it can help in developing an algorithm of informative markers for evaluating chimerism after allogeneic BMT.^{19,20} If these three STR markers are not informative in a patient, the next three

can be checked in the patient donor pair i.e. D7S820, TH01, FGA. Furthermore, the commonly used STR markers can also be set together in a multiplex PCR to yield cost effective results.

The patients in the study were predominantly males (61.9%); the female underrepresentation could be because of gender bias in our society when seeking costly medical attention for different diseases. Secondly, no study shows gender as a prognostic marker for post-transplant outcomes in either aplastic anaemia or beta-thalassaemia. In this study also, the percentage of males and females achieving MC was 59.6% and 56.3%, which is not a major difference.

In this study, the frequency of MC in patients of non-malignant haematological diseases at mean post-transplant duration of 8.8 months was 58.3%. Since this study is the first of its kind in Pakistan; therefore, no local studies were available on the subject for purpose of comparison of these results despite thorough search in the literature. Many foreign studies assessing donor chimerism in the patients of either beta-thalassaemia or aplastic anaemia alone have been done. Majority of these studies follow the patients in the post-transplant period and sequentially monitor donor chimerism to assess the state of haemopoiesis. Additionally, no exact definition for MC was available; which could be one of the reasons for an increase percentage of MC. Most of the present patients had > 50% MC with a mean percentage of donor origin DNA of 86.3% and 90.9% in aplastic anaemia and beta-thalassaemia major respectively.

Lisini *et al.* showed that out of the 79 patients of beta-thalassaemia major, 51 (64.5%) exhibited MC and 24 (30.3%) developed CC which is comparable to results of this study, where out of the 50 post-transplant patients of beta-thalassaemia, 31 (62%) achieved MC and 13 (26%) developed CC. However, in an Italian study by Andreani *et al.*, the incidence of MC in 93 patients of beta-thalassaemia major who underwent allogeneic BMT was 46.2% (n = 43) while that of CC was 53.7% (n = 50). The difference in results could probably be due to the mean duration of assessing chimerism in study by Andreani *et al.* was between 20 days to 60 days, whereas in this study, it is 8 months. The study by Andreani *et al.* also highlighted that the percentage of donor cells may decrease with subsequent follow-up leading to graft rejection, if at initial assessment, the percentage of residual host cells was > 25%.²¹

In a large study done by Huss *et al.* on 116 post-BMT patients with aplastic anaemia, who received stem cells from HLA-identical siblings, MC was seen in 63 (54%) patients. This is comparable to that of our study, where MC was seen in 50% of aplastic anaemia patients. In an older study by Hill *et al.*, almost 58.3% of the patients of aplastic anaemia had mixed chimerism after allogeneic

BMT. Here again, the difference may be because the chimerism was assessed between post-transplant days 14 and 100.

Some of the limitations of the study were that the sampling technique was non-probability sampling that could affect the results. Additionally, the factors affecting engraftment and post-transplant chimerism like exposure to blood components, disease severity, stem cell dose, donor and transplant related characteristics e.g. blood group mismatch and gender mismatch were not available.

Local studies pertaining to mixed donor chimerism in patients of allogeneic BMT are virtually non-existent in Pakistan, whereas the number of transplant centre continues to rise in the country. Therefore, a lot of work needs to be done in this regard. In the future prospective studies that follow the patients in a longitudinal manner and assess the degree of mixed donor chimerism at 3 months, 6 months and 12 months need to be carried out. Also the correlation of mixed donor chimerism with the blood counts of the patient at these intervals may provide a clue, as to which haematological parameter of blood counts is best indicative of a higher mixed donor chimerism percentage.

CONCLUSION

Following a BMT for non-malignant haematological diseases majority of the patients achieve mixed donor chimerism. Analyses of D3S1358, D5S818 and D8S1170 STR markers are most informative in these patients. However, an isolated analysis of donor chimerism percentage is not sufficient and serial chimerism analyses over a period of post-transplant duration are required for better interpretation of the donor haematopoiesis in the recipient.

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