IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 4 Ver. 18 (April. 2018), PP 20-23
www.iosrjournals.org

Study of Chimerism in Haematopoietic Stem Cell Transplant Recipients: An Experience at Army Hospital (Research & Referral), Delhi Cantt

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Abstract
Background: *Allogeneic Haematopoietic Stem Cell Transplant (HSCT) is panacea of cure for various malignant and non-malignant haematological conditions in medicine today. Chimerism study is a valuable tool in monitoring graft outcomes in allo-HSCT recipients. This study outlines usefulness of chimerism studies as predictor of graft rejection or disease relapse in cases with mixed chimerism and also as surrogate marker of clinical and haematological variables in these patients.*
Methods: *A total of 57 recipients of allo-HSCT were followed up with chimerism study as per protocol. Multiple short tandem repeat (STR) amplification using fluorescence labelling polymerase chain reaction (PCR) combined with capillary electrophoresis was the method employed.*
Results: *Nine allo-HSCT recipients showed mixed chimerism at various times during follow-up. Outcome in these cases was variable with five patients dying during the study. In the cases with mixed chimerism, chimerism status was found to be useful surrogate marker of clinical and haematological variables predicting rejection or relapse of disease.*
Conclusion: *Study of chimerism status in allo-HSCT recipients is a valuable predictor of outcome of grafts rejection or disease relapse. It may often predate the clinical tremulousness in patients and provide the managing team with precious lead time.*
Key Words:
Chimerism Analysis,Haematopoietic Stem Cell Transplantation,Short Tandem Repeats,Polymerase Chain Reaction

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Date of Submission: 16-04-2018 Date of acceptance: 02-05-2018

I. Introduction

In the medical field "chimera" is an exceptional immunogenetic and biologic state in which cell populations originating from another individual of the same or different species are living, differentiating and functioning. The chimeric state is naturally achieved in recipients of HSCT and solid organ transplant[1]. Studies about finding the chimerism status in transplant recipients has the potential of detecting risks of rejection of the graft , Graft vs. Host Disease(GVHD) or Graft vs. Leukaemia (GVL) effect well before the clinical manifestations. Therefore, it can provide the clinician with reasonable lead time for appropriate management measures.

II. Aim & Objective

The aim of this study was to analyze monitoring of allogeneic hematopoietic stem cell transplant patients through chimerism analysis and predict the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT).

III. Material and Method

Multiple short tandem repeat (STR) amplification using fluorescence labelling polymerase chain reaction (PCR) combined with capillary electrophoresis was the method used. Peripheral blood of 57 patients who received allo-HSCT was collected before and after transplantation as per protocol. 16 different STR markers were amplified in multiplex PCR by using a commercially available *AmpF/STR Profiler Plus* PCR amplification kits. Separation of the PCR products and fluorescence detection were performed by ABI prism 3100 Genetic Analyzer. The Genemapper V 4.0 software was used for size calling and quantification of peak

areas. The formula to calculate donor chimerism values was based on the different allelic distribution type between donor and recipient.

IV. Result

1. The study included a cohort of 57 patients with 42 males and 15 females. Spectrum of age was 1-52 yrs.
2. Both malignant and non-malignant groups of cases were included and consisted of 27 patients with non-malignant haematological conditions and 30 patients belonging to malignant group of haematological diseases. Distribution of diseases and status of chimerism as assessed on different occasions (D+n) are shown in Table-1.
3. Forty-eight cases showing complete chimerism through the study period did not show any feature of relapse of their primary disease or graft rejection.
4. Nine out of 57 patients showed mixed chimerism detected on different days of their follow up as per protocol.
5. Four out of 9 cases showing mixed chimerism died due to graft rejection, primary disease or related complications and were lost to follow up.
6. The other five were surviving and showing varying degrees of graft acceptance. The recent most follow up of these patients had been within a month prior to this study.
7. In all cases showing mixed chimerism the detection of chimerism status predated the deterioration of their clinical condition.
8. Increase or decrease in values of mixed chimerism (Table-2) in the five surviving cases was a good predictor of improving or deteriorating clinical condition respectively, of these patients.
9. The values of mixed chimerism also showed good correlation with haematological values like Haemoglobin (Hb), Total Leukocyte Count (TLC) and platelet counts of the patients done on each occasion along with testing of chimerism status.
10. The correlation of chimerism values with total leukocyte count of one patient has been depicted in Chart-1.

V. Discussion

Different types of chimerism are known including complete, mixed or split chimerism. There are various methods used to detect the type of chimera state depending on the immunogenetic differences between the donor and recipient. Patients who show no evidence of host DNA at any time during the post transplant follow-up are considered to be in "Complete Chimerism" (CC). CC is usually accepted to be associated with a low risk of relapse and better prognosis. Patients with donor and recipient DNA in any of the samples were defined as having mixed chimerism (MC). MC indicates the presence of both donor and recipient cells within a given cellular compartment[2]. The term split chimerism, may be used when one or more compartment is derived wholly from the donor. The different types of chimerism developing after HSCT and peripheral blood stem cell transplantation (PBSCT) may be parallel with the prognosis of the disease. Thus, mixed chimerism associates with less graft versus host disease (GVHD) and graft versus leukaemia (GVL) effect, higher frequency of relapse and shorter disease- free survival[3]. In contrast, complete chimerism relates to a more frequent and more severe GVHD, less relapse and longer disease-free survival.

Analysis of chimerism by PCR based methods aimed at precise differentiation between the donor and recipient cells are now widely employed in molecular technology across the world. Techniques using short tandem repeats polymorphisms of DNA have proved by far to be one of the most sensitive methods for studying chimerism in allo-HSCT cases and are the preferred method in clinical settings[4]. These methods are relatively rapid and circumvent the problem of very few cells being present for analysis[5].

Allogeneic haematopoietic stem cell transplant is the paradigm of therapy for many haematological diseases which till few years back had no cure.However, the prognosis and clinical course of recipients is unpredictable by routine clinical and investigational parameters. Chimerism analysis has proven to be an important cornerstone to change this uncertainty to a remarkable extent. The outcome in patients showing mixed chimerism is variable as this depicts relatively poor graft acceptance by the recipient and is possibly a result of many well established and some yet undefined factors[6]. The well defined factors affecting outcome of allo-HSCT engraftment include effective immunomodulation both extrinsic and intrinsic; host's leukocyte depletion; the quantum of stem cells infused; timely and efficient management of infections; genetic compatibility between donor and recipient blood cells and the inherent sensitivity and timing of chimerism testing[7]. Detection and timely monitoring of the mixed chimerism status in these patients has proven to be useful in appropriate management initiations or modifications in order to prevent graft rejection, relapse of disease and graft versus host disease[8]. The chimerism values may be reliable surrogate markers for this. Graft recipients with mixed chimerism need to be followed up carefully and for a longer period of time to demonstrate a satisfactory graft acceptance and to monitor disease relapse[9]. Further studies with larger cohorts of cases is definitely a way

forward to be able to define different viable correlations between chimerism values and allo- HSCT graft outcomes[10].

VI. Conclusion

Chimerism study in allo-HSCT graft recipients is a useful tool to monitor outcomes in these patients. Complete chimerism is a predictor of good graft outcome with usually successful graft acceptance by the recipient. Mixed chimerism, on the other hand, correlates with variable graft outcomes and needs to be closely monitored by sensitive methods for chimerism values of graft recipients and for a longer duration of time[11]. Variations in the values of chimerism in cases with mixed chimerism usually correlate well with clinical and haematological condition of graft recipients. A more diligent and cautious approach is required to monitor mixed chimerism status and clinical condition in these patients to achieve satisfactory allo-HSCT engraftment.

Intellectual contribution of authors
Study concept: Col M S Bindra
Drafting and manuscript revision: Col M S Bindra, Col Sunil Arora
Statistical analysis: Col M S Bindra, Col Sunil Arora
Study supervision:Col M S Bindra
Conflicts of Interest:
All authors have none to declare.

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Table 1: Data showing status of chimerism at various time intervals

S No	Diagnosis	No of cases		D+30	D+60	D+90	D+120	D+180	D+270	D+360
1.	Thalassemia major	14	CC	10	10	10	10	10	10	10
			MC	04	04	03	03	01	-	-
2.	AML	13	CC	13	13	13	13	13	13	13
			MC							
3.	ALL	9	CC	09	09	09	09	09	09	09
			MC							
4.	Aplastic anaemia	7	CC	06	06	06	06	06	06	06
			MC	01	01	01^				
5.	CML	4	CC	03	03	03	03	03	02	02
			MC	01	01	01^				

	MDS	4	CC	04	04	04	04	04	04	04
			MC							
7.	Fanconi anaemia	2	CC	02	01	01	01	01	01	01
			MC		01	01^				
8.	PNH	1	CC	01	01	01	01	01	01	01
			MC							
9.	CDA	1	CC	01	01					
			MC			01	01	01	01	-
10.	Others	2	CC	01	01	01	01	01	01	01
			MC	01	-					

ALL-Acute Lymphoid Leukaemia; AML-Acute Myeloid leukaemia; CDA-Congenital Dyserythropoietic Anaemia; CML-Chronic Myeloid Leukaemia; MDS-Myelodysplastic Syndrome; PNH-Paroxysmal Nocturnal Haemoglobinuria; CC-Complete chimerism; MC-Mixed chimerism; ^-Death;(Others –Pure Red Cell Aplasia & X-linked myelodystrophy)

Table2: Changes in the percentage of chimerism in surviving case

Sr No.	D+30	D+60	D+90	D+120	D+180	D+270	D+360
1.	79.45%	82.00%	76.80%	-	70.55%	-	59.16%
2.	100%	100%	68.6%	40.05%	29.21%	32.28%	-
3.	100%	72.06%	41.99%	71.26%	55.70%	38.7%	43.50%
4.	100%	54.30%	90.0%	81.5%	-	-	-
5.	36%	-	93%	89.2%	-	-	-

D-Day of transplant, % of chimerism status.

Chart 1: Distribution of patients as per disease

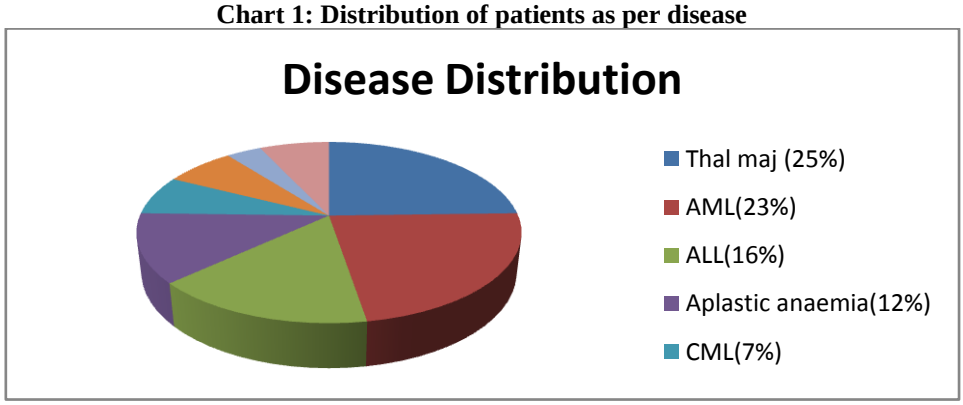

Chart 2: Correlation of percentage of chimerism with total leukocyte count