Section: Transfusion Medicine

Original Research Article

NON-CRYOPRESERVED PERIPHERAL BLOOD STEM CELL STORED AT 2°C TO 4°C FOR 4 DAYS IN AUTOLOGOUS TRANSPLANT: LONGITUDINAL STUDY

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ABSTRACT

Background: Haematopoietic stem cell transplantation remains the only curative option for many haematopoietic malignancies. The pluripotent haematological stem cell required for this procedure are usually obtained from the bone marrow or peripheral blood. Currently majority of procedures for procurement of haematopoietic progenitor cells are performed by peripheral blood apheresis collection. Objective: To assess the Viability of Peripheral blood Haematopoietic stem cell after storage in 20 to 40c for 4 Days. **Materials and Methods:** During the study period all patients who underwent Autologous HSCT procedure for Hematological malignancies was included and the related factors were obtained. Totally 12 Autologous HSCT patients are mobilised with G-CSF and one patient with Plerixafor and collected by apheresis. Under aseptic precaution 3ml of stem cell product from the stem cell bags were collected and aliquot in to two sample with proper labelling DAY 0 and DAY 4. Day4 sample was kept in 2-40c in blood bank refrigerator and for analysis the viability of CD34 cells on day 4 from the collection date. **Results:** In our study the viability of CD34 cells peripherally collected blood stem cells which stored at 2oc to 4oc up to 4 days show viability in range of (82%-92%). **Conclusion:** In developing countries like India, establishing Bone Marrow Transplantation centre in Public Health Care System is a rarity. This is because of the cost involved and extremely special care to be given for successful outcome. Further, the infrastructure needed for cryopreservation of stem cells adds up the burden on healthcare providers.

INTRODUCTION

Hematopoietic stem cell transplantation remains the only curative option for many hematopoietic malignancies. The pluripotent hematological stem cell required for this procedure is usually obtained from the bone marrow or peripheral blood. Currently, the majority of procedures for procurement of hematopoietic progenitor cells are performed by peripheral blood apheresis collection.

The development of apheresis technology, the discovery of hematopoietic growth factors and small molecule CXCR4 antagonist for stem- cell mobilization, and in vivo experimental transplantation studies eventually led to clinical Peripheral Blood Stem Cell Transplantation.

The minimum number of cells needed for transplantation is commonly cited as 2×106 CD34+ cells/kg, although 5×106 CD34+ cells/kg is more desirable.

The advantage of early engraftment of hematopoietic stem cell transplantation includes, reduced incidence of post-transplant neutropenia associated infections, mortality, morbidity and shorten the length of hospital stay that eventually reducing the overall cost However, the conventional procedures for the collection and freezing of PBSCs are time-consuming and expensive. Methods to simplify bone marrow transplantation procedures are needed mainly in developing countries.

MATERIALS AND METHODS

This Longitudinal Study was conducted among Hemato-Oncological Patients undergoing autologous hematopoietic stem cell transplantation at Dept of hematology, RGGGH & MC, Chennai, Tamil Nadu from November 2020 To October 2021. The study was approved by both university and intuitional ethics committee.

Sampling Technique: Purposive Sampling Technique

Sample Size

Number of cases admitted for Autologous Haematopoietic stem cell transplantation during the study period

Inclusion Criteria

Haemato-Oncological Patients Eligible for autologous hematopoietic stem cell transplantation

Exclusion Criteria

Allogenic stem cell transplant patients and Eligible but not willing to participate in the study are excluded.

Informed Consent

The study details were completely explained to the patient's relatives who were included in this study at the Dept of Haematology, Madras Medical College and Hospital, Chennai.

Statistical Method

The collected data were analysed with IBM.SPSS statistics software 23.0 version. To describe about the data descriptive statistics, frequency analysis, percentage analysis were done. chi square test for association, frequency table and graphical representation were done.

RESULTS

Frequency distribution age group in our study population Age group 12-24 (8.3%) 24-36 (8.3%) 36-48 (41.7%) >48years (41.7%).

Table 1: Cross-tabulation of Age Group x Cell dose 10^6/Kg

Cell dose 106/Kg on Day0	Age Group				Total	Chi-square value	p- value
	12-24	24-36	36-48	>48			
4x10^6	0	1	1	5	7	20.23, df=3	0.017
5x10^6	0	0	1	0	1		
6x10^6	0	0	3	0	3		
8x10^6	1	0	0	0	1		
Total	1	1	5	5	12		

Since the p-value is less than 0.05, there is no evidence to accept the null hypothesis. Therefore, there is an association between Cell dose 106/Kg on Day 0 and Age group.

The above table shows that a total of 12 cases 4-8x106 hematopoietic cell dose obtained out of 12 cases the age group (12-24) has obtained 8x106 and age group >48 years obtained 4x106

In our study group 75% was male gender and 25% was female gender.

Table 2: Cross-tabulation of Cell dose 10^6/Kg on Day0 * Gender

Cell dose 10^6/Kg on Day0	Gender		Total	Chi-square value	p-value
	Male	Female			
4x10^6	4	3	7	2.857 and df=3	0.414
5x10^6	1	0	1		
6x10^6	3	0	3		
8x10^6	1	0	1		
Total	9	3	12		

Since the p-value is greater than 0.05, there is no evidence to reject the null hypothesis. Therefore, there is no association between Cell dose 106/Kg on Day 0 and gender.

Table 3: Frequency distribution of Diagnosis

Diagnosis	Frequency	Percent
MM	6	50.0
HL	4	33.3
NHL	1	8.3
NHL/DLBCL	1	8.3
Total	12	100.0

The above table shows the frequency distribution of diagnosis in our study group out of 12 patients 6 were Multiple myeloma 50% and 4 patients were Hodgkin's lymphoma 33% 1 patient were Non-

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Hodgkin lymphoma 8.3% and 1 patient were NHL-DLBCL 8.3%

Out of 12 patients 41% patients not associated with any comorbidity,25% patient associated with DM with HCV+,16.7% patients associated with CKD,8.3% patients associated with HBsAg,8.3% patient associated with HCV alone.

Table 4: Cross-tabulation of Gender and G-CSF

Gender	G-CSF		Total
	D5	D7	
Male	9	0	9
Female	0	3	3
Total	**9**	**3**	**12**

The above table shows that Cross-tabulation of Gender and GCSFf shows out of 12 patients 9 patients are male and 3 patients are female in this only female patient need G-CSF for 7 days to mobilize adequate cd34 cell dose.

Table 5: Cross-tabulation of Cell dose 10^6/Kg and Plerixafor

Cell dose 10^6/Kg on Day0	Plerixafor		Total
	Not Needed (NN)	Needed	
$4x10^6$	6	1	7
$5x10^6$	1	0	1
$6x10^6$	3	0	3
$8x10^6$	1	0	1
Total	11	1	12

The above table Cross-tabulation of Cell dose 10^6/Kg and Plerixafor shows out of 12 patients 7 patient have less than $5x10^6$/kg cells in that 1 patient need plerixafor.

Table 6: Cross-tabulation of pre peripheral blood cd34 count group and Plerixafor

Peripheral blood cd34 count	Plerixafor		Total
	Not Needed (NN)	Needed	
<10	0	1	**1**
>10	11	0	**11**
Total	**11**	**1**	**12**

The above table Cross-tabulation of pre peripheral blood CD34 count group and Plerixafor shows that out of 12 patients 11 patients pre peripheral blood CD34 count more than 10 and 1 patient had pre peripheral blood count less than 10 and that patient needs plerixafor for mobilization of CD34 cell for adequate cell dose.

Table 7: Cross-tabulation of Cell dose 10^6/Kg on Day0 * Diagnosis

Cell dose 10^6/Kg on Day 0	Diagnosis				Total	Chi-square value	p-value
	MM	HL	NHL	NHL/DLBCL			
$4x10^6$	2	3	1	1	7	7.43 and df=9	0.593
$5x10^6$	1	0	0	0	1		
$6x10^6$	3	0	0	0	3		
$8x10^6$	0	1	0	0	1		
Total	6	4	1	1	12		

Since p-value is greater than 0.05, there is no evidence to reject the null hypothesis. Therefore, there is no association between Cell dose 106/Kg on Day 0 and diagnosis.

The above graphical representation shows in our study 12 samples of peripheral blood hematopoietic stem cells collected and stored for up to 4 days. The Day 0 sample CD 34 count is (99%-100%) and the Day 4 CD34 sample (82%-92%. [Figure 1]

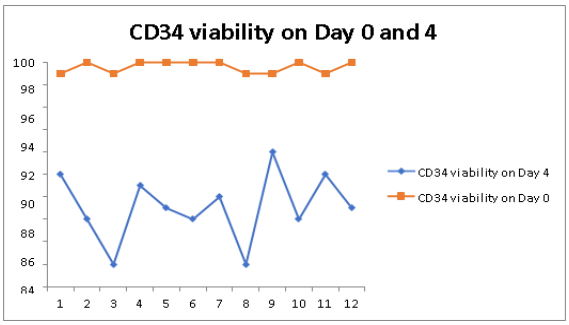

Figure 1: CD34 cell viability on Day 0 and Day 4

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DISCUSSION

Table 8:

Research	Noncryopreservation method	Cd34 cell viability	Stored at 2-4° c	Autologous Transplant
Our study (2020-2021)	Noncryo method	(80%-92%)	4 days	AT
Mohamed- Amine Bekadja et al (2021) [1]	Noncryo method	(74%-97%)	6 days	AT
M. Sarmiento et al (2018)[2]	Noncryo method	(92%-96%)	6 days	AT
Amado Kardduss-Urueta et al(2018) [3]	Noncryo method	88%	(1-6days)	AT
Smita Kayal, Atul Sharma et al(2014) [4]	Noncryo method	88%90%	(1-5days)	AT
Uday Kulkarni et al (2018) [5]	Non cryo method	NA	Up to 72hrs	AT
G. Hechler et al(1995) [6]	Noncryo method	80%	5-8days	AT

In our study, the main aim is to assess the viability of non-Cryopreserved peripheral blood stem cells stored at 2-4oc after 4days. The study included 12 autologous stem cell donors ailing from haemato-oncological diseases. There were 9 male and 3 female donors, ages ranging from 27 to 64. The study population is similar to Smita Kayal, Atul Sharma et al, however, the duration of the study is more than 5 years, and several donors studied were ranging from >90 patients in SmiKayalyal, Atul sharma et al, studies included allogenic donors also.

The CD34+ cell count and CD34+ Cell viability were done by flow cytometry, on Day 0 and Day 4. In our study, the Day 0 CD34 cell viability was in the range of 99 to 100%, on Day 4 it was in the range of 82 to 92%. This is similar to G. Hechler, R. Weide et al studies.

The CD 34+ cell count and CD34 cell viability after post-thawing study done by S. LEE, S.KIM, et al, after thawing cryopreserved stem cells was reduced to 71% (range 31%-89%). There was a significant reduction in the viability of CD 34 cells, while the cryo-preserving and thawing procedure.

This infers selective cases for cryopreservation. Moreover, the side- effects of DMSO need to be considered. The method of non-cryopreservation for short conditioning regime can be considered as the preferred option. This is cost-effective and almost equally effective with the preservation of a requisite number of viable CD34+ stem cells.

The duration between Day 0 and Day 4 can be utilized for short conditioning regimen, as in the case of Multiple Myeloma treated with melphalan

The outcome of engraftment was successful after infusion of non- cryopreserved stem cells in various studies referred to in Table8.

Since there were no previous studies done in our bone marrow transplantation center, our study was limited at the level of assessing CD 34+ viability alone.

CONCLUSION

Serum AMH levels were more robustly correlated with AFC than serum FSH, LH, E2 on day 3 of cycle. AMH and AFC correlate significantly with the number of oocytes retrieved and hence serve as better predictors of ovarian response to ovarian stimulation with exogenous gonadotropins using the GnRH antagonist protocol. AMH also serves as a good predictor of the dose of gonadotropins during stimulation before starting an IVF cycle. This suggests that AMH, a new marker, may reflect ovarian function better than usual hormone markers.

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