Improving Outcome of Thalassemia with Hematopoietic Stem Cell Transplantation: An Experience of Gujarat Cancer Research Institute

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Received: 24 January 2015 / Accepted: 25 July 2015 / Published online: 2 August 2015
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Abstract Total 26 children of thalassemia underwent hematopoietic stem cell transplantation from September 2006 to December 2014. Out of these 17 were matched sibling transplantation (MST) and 9 were unrelated umbilical cord blood transplantation (UCT). Median age was 4 years. At a median follow up of 46.5 months, 12 of 17 (70 %) MST and 3 out of 9 (33.33 %) UCT were cured of thalassemia. Three (11.53 %) patients died due to transplant related mortality. Average cost of MST was 6 lakhs and that of UCT was 20 lakhs.

Keywords Thalassemia · Allogenic hematopoietic stem cell transplantation · Matched sibling · Umbilical cord

Introduction

Allogenic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for thalassemia. Its success depends on risk category which is based on hepatomegaly, portal fibrosis and inadequate pretransplant iron chelation therapy.

Pesaro Risk class: Based on Hepatomegaly, portal fibrosis and high ferritin levels

- Class I—no adverse factor
- Class II—1–2 adverse factor
- Class III—3 adverse factors

Patients and Methods

Design Retrospective, **Setting:** Gujarat cancer research institute (GCRI), Ahmedabad; **Period:** September 2006 to December 2014.

Table 1 specifies risk stratification of patients indicating that majority of the patients were moderate to high risk.

Transplant protocol Baseline blood investigations: Complete blood counts, biochemistry, HIV, HbsAg, HCV, pulmonary function test and 2-D Echocardiography.

Preparation

Donors for matched sibling transplantation (MST) received granulocyte colony stimulating factor (G-CSF) 10 mcg/kg every 12 hourly total 9 doses before stem cell collection. Target for stem cell collection was greater than 10×10^8 MNC/kg and target for CD34 in collection was $2-5 \times 10^6$ /kg.

Search of cord blood for umbilical cord transplantation (UCT): Searches for Umbilical cord blood units were done through the StemCyte Cord Blood Bank, Taiwan and Relicord (reliance life sciences) cord blood bank, India. At least 4 out of 6 antigens within HLA-A, -B, and -DR B1 matched (serologic or intermediate-DNA level of resolution) umbilical cord blood units selected. The compatibility of HLA-A, -B, and -DRB1 was further confirmed by high-resolution polymerase chain reaction with sequence-specific primers (PCR-SSP). HLA disparity between each unit and the recipient and between two units did not necessarily have to be at the same loci.

Procedure

Written and informed consent of all patients and their relatives is taken explaining the risk and benefits of

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Table 1 Risk stratification of patients

Pesaro class	Matched sibling HSCT (N)	Partially matched unrelated umbilical cord HSCT (N)
Class 1	2	4
Class 2	7	5
Class 3	8	2

The table shows that most of the thalassemia candidates for HSCT were intermediate and high risk class

transplants. All the patients are admitted to bone marrow transplantation (BMT) unit before starting conditioning regimen. The BMT unit is provided with high efficiency particulate air (HEPA) filters.

Graft source: It included 14 peripheral blood stem cell (PBSC) sources, 3 bone marrow (BM) sources and 9 unrelated umbilical cord blood (UCB) sources.

Matched sibling transplant (MST) was with 6/6 match.

In UCT maximum 2 mismatches were allowed. Five of UCT patients had single donor while 4 patients had double donor graft source. The mismatches in double graft sources were not necessarily at same locus.

Preferred cell dose for BM/PBSC: $2-5 \times 10^8$ MNC/kg.

Preferred cell dose for cord blood: 10×10^7 TNC/kg.

Conditioning Regimen

We used myeloablative conditioning regimen in all patients. Reduced toxicity regimens (RTC) were not used. Table 2 describes conditioning regimens. Busulfan or Treosulfan, Cyclophosphamide and antithymocyte globulin (ATG) was used as conditioning regimen for MST while Fludarabine was added for conditioning regimen for UCT. G-CSF was used from day +1 for UCT patients.

End points: Primary endpoints to study were engraftment, graft failure, graft versus host disease (GVHD) and transplant related mortality (TRM).

Engraftment Granulocyte engraftment was defined as 3 consecutive days with absolute neutrophil count (ANC)

$>0.5 \times 10^9/L$ or 1 day with a count of $>1 \times 10^9/L$. Platelets engraftment was defined as platelet count $>20 \times 10^9/L$ on 3 consecutive days.

Chimerism analysis: It was done on day +30 using variable number of tandem repeats (VNTR) analysis in same sex recipient- donor and with Fluorescent in situ hybridization (FISH) in different sex recipient-donor.

Graft failure It is suggested by decreasing hemoglobin or persistent requirement of blood transfusion and also by chimerism study.

Antimicrobial prophylaxis: For all patients it included

- Isolation till day +30 post transplant.
- Oral cavity, skin and perianal hygiene.
- Tablet Albendazole 400 mg single dose on day −9.
- Tablet Acyclovir 200 mg TDS from the start of conditioning till approx 30 days.
- Tablet Cotrimoxazole DS alternate day from −14 to day −2 then from engraftment till 6 months or till GVHD resolves whichever is later.
- Inj Ganciclovir 5 mg/kg BD—Preemptive.
- Single donor platelet (SDP) if Platelet count (PC) is $<20 \times 10^9/L$ and Packed red cells (PCV) if haemoglobin (HB) is <8.0 gm %.

GVHD Prophylaxis

MST candidates received prophylaxis with cyclosporine (5 mg/kg), and methotrexate while UCT patients received

Table 2 Conditioning regimens for MST and UCT

Conditioning regimens for matched sibling transplant (MST)

BCA (N = 15) i.e. Busulphan (0.8 mg/kg/dose $\times$ 16 doses), Cyclophosphamide (200 mg/kg divided in 4 days), ATG (30 mg/kg/day $\times$ 3 days) or

TCA (N = 2) (Treosulfan substituted for busulfan)

Conditioning regimen for unrelated umbilical cord transplant (UCT)

FBCA i.e. Fludarabine (30 mg/m²/dose $\times$ 3 days), Busulphan (0.8-1 mg/kg $\times$ 16 doses), Cyclophosphamide (200 mg/kg divided in 4 days)

ATG (30 mg/kg/day $\times$ 3 days)

Granulocyte colony stimulating factor (GCSF) was started from day 0 for unrelated umbilical cord transplant patients

Conditioning regimen for retransplantation

Cyclophosphamide 120 mg/kg (day −3 to −2)

The table shows the conditioning regimens for MST and UCT

prophylaxis with mycophenolate (15 mg/kg/dose) and cyclosporine (5 mg/kg).

Statistical analysis It was carried out using SPSS 20 software.

GVHD Acute GVHD is defined as that between 30 and 100 days and chronic GVHD as that after 100 days.

Results

Table 3 shows gross analysis of the patient outcome.

Total 26 children of thalassemia underwent HSCT in the study duration. Out of these 17 were MST and 9 were

UCT. Twenty (77 %) were males and six (23 %) were females. Median age was 4 (1–9) years.

In MST 1 patient showed primary graft failure while 16 showed engraftment. Further 3 of these 16 patients had TRM and 1 showed autologous recovery on day +60. Thus 12 MST were successful.

In UCT, 5 patients showed engraftment but 2 of these further showed autologous recovery on day +60. Thus 3 UCT were successful.

At a median follow up of 46.5 months 12 of 17 (70 %) MST and 3 out of 9 (33.33 %) UCT were cured of thalassemia. Overall 3 (11.5 %) patients were died due to TRM. All fatalities were in high risk MST patients. In

Table 3 Analysis of Thalassemia patients at GCRI (medians of variables)

	Pbse + BM	UCT	Overall
Method	14 PBSC, 3 BM	9	26
Age in years	4 (1–9)	2 (1–7)	4 (1–9)
Total patients	17	9	26
Conditioning regimen	BCA in 15, TCA in 2	FBCA	
Dose of cells	4.345×10^8 MNC/kg	10.71×10^7 TNC/kg	
Successful engraftment ANC >500, untransfused PLC >20,000	16/17 (94.11 %)	Initialiy 5/9 (56 %) patients showed engraftment and 4/9 (44 %) had autologous recovery	21/26 (80.76 %)
Disease recurrence Loss of chimerism,decreasing HB, requirement of blood transfusion	1/16 (0.0625 %) patients showed autologous recovery on day +60	2/5 (40 %) patients showed autologous recovery on day +60	3
Transplant related mortality (100 days mortality)	3 (all with successful engraftment; 1 due to infection, 1 due to GVHD and 1 due to hematuria)	0	3
GVHD	6/16	4	10/21
Skin	2	1	
Liver	2	1	
Skin + liver	2	2	
<i>Note</i> 1 skin GVHD was grade 4, rest all limited grade			
GVHD	6	4	10/21
ACUTE	1	3	
CHRONIC	5	1	
Follow up months	48 (21–113)	45 (32–75)	46.5 (21–113)
Disease free follow up patients at this follow up	12 Patients	3 Patients	15 Patients
Neutrophil engraftment day	+15	+17	+16
Platelet engraftment day	+17	+36	+23
Oral cyclosporine started at +day	+18	+22	+20
Rbc units required	5	5	5
SDP units required	13	14	13.5
Hospitalization post stem cell infusion days	+35	+36	+35

The table shows the gross characteristics of HSCT candidates

MST, no patient had rejection mortality but all 3(17.64 %) patients had non rejection mortality, 1 due to infection, 1 due to GVHD and 1 due to hematuria. In UCT, there was no TRM.

Table 4 shows the engraftment for MST in relation to conditioning regimen, stem cell source and stem cell dose. Though the data shows early engraftment with treosulfan, the sample size with treosulfan containing regime was very small. PBSC source shows early engraftment than BM source. Also, higher the stem cell dose, earlier the engraftment.

Table 5 shows the comparison of indian and international outcomes in thalassemia transplantation. This indicates that in MST, disease free survival is poor in high risk cases in Pesaro study(60 %) as well as CMC vellore(55 %) and same is reflected in our study also (DFS with MST in high risk 50 % and DFS with UCT in both moderate and high risk being 0 %). Thus the risk classes is the uniform predictable parameter irrespective of institutes and techniques of transplantation. Second transplantation was done for only two failed UCT patients which again showed graft failure. Some other indian institutes have not reported risk class wise outcomes, hence it is difficult to compare with their outcomes.

Table 6 shows the graft specific results of MST. Though the data shows that the outcome for PBSC in engraftment and TRM was better than BM source, it is to be noted that the sample size of BM graft sources are less in this study. Even though BM being the standard source in thalassemia patients, due to ease of collection PBSC source was preferred and outcome with PBSC showing 100 % engraftment with only 14 % TRM is encouraging and justifies the recent trend of PBSC for stem cell collection.

Table 7 shows the incidence of GVHD with graft source and conditioning regimens. Two of 3 patients with BM graft source had GVHD (66.66 %), 1 being acute GVHD (33 %) which had TRM and 1 being chronic GVHD (33 %). For PBSC, none of the patients had acute GVHD

(0 %) and 4 (28.5 %) patients had chronic GVHD. For comparing conditioning regimens with GVHD incidence, the sample size of TCA regimen was very small. Patients on BCA regimen noticed 1 acute GVHD (6.66 %) and 4 chronic GVHD (26.66 %).

Table 8 shows cost effectiveness of HSCT thalassemia treatment. The expenses to manage a thalassemia patient without HSCT are virtually unlimited and infinite (approximately 2 lakh rupees per year for life time). While the average cost of MST was 6 lakh and that of UCT was 20 lakh but these are limited and if the HSCT is successful, it is just for one time. Also without HSCT the patient is morbid for lifetime and in HSCT the morbidity is limited to the risk of procedure. If the procedure related risk is minimized with involvement of experienced transplant physician, HSCT should be the treatment of choice in all thalassemia patients.

Engraftment (MST versus UCT): Sixteen out of 17(94.11 %) MST showed engraftment while 5 out of 9 (56 %) UCT showed engraftment. For MST granulocyte engraftment occurred on day +15 and platelet engraftment occurred on day +17. For UCT granulocyte engraftment occurred on day +17 and platelet engraftment occurred on day +36.

GVHD (MST versus UCT): Six out of 16 (37.5 %) engrafted MST patients had GVHD. Two patients had skin GVHD, 2 had Liver GVHD and remaining two had both skin and liver GVHD. Only 1 skin GVHD was grade 4. Rest other GVHD were limited grade. In UCT patients 3 patients had acute GVHD and 1 patient had chronic GVHD, all being in limited grade.

Survival, deaths and persistent transfusion dependence: Overall TRM was 3 (11.5 %), 15 out of 26 (57.69 %) patients were cured (transfusion free) and remaining 8 (30.76 %) were persistent transfusion dependant due to thalassemia. TRM due to MST was 17.64 % (all due to infection) and that due to UCT was 0 %.

Table 4 The engraftment in relation to conditioning regimen, stem cell source and stem cell dose in MST patients

	Median granulocyte engraftment day (+)	Median platelet engraftment day (+)
Conditioning regimen		
BCA (N = 15)	15	17
TCA (N = 2)	14	16
Stem cell source		
PBSC (N = 14)	14	16
BM (N = 3)	16	18
Stem cell dose × 10 ⁸ MNC/Kg		
≤2.4	19	23
≤5	16	19
>5	14	16

Table 5 Disease free survival (DFS) in Pesaro—Italy (1997) analysis versus GCRI (2014) analysis along with other Indian reports for MST in thalassemia

Class	Pesaro	CMC vellore	Eastern India	Northern India	GCRI (PBSC + BMT)	GCRI (Cord)
1	93 %	66 % (N = 6)	N = 6	(Class 2 = 7; Class 3 = 21)	100 % (N = 2/2)	75 % (N = 3/4)
2	85 %	90 % (N = 30)	DFS = 33.33 %	DFS = 71.4 %	85.71 % (N = 6/7)	0 % (N = 5/5)
3	60 %	55 % (N = 70)			50 % (N = 4/8)	0 % (N = 0/2)
Second transplantation		25 % (N = 4)	NA	NA	NA (N = 0)	0 % (N = 2)
Median follow up		26 months	4.6 years	387 days	46.5 months	46.5 months

The table shows the comparison of Indian and international outcomes in thalassemia transplantation

Table 6 Outcome of matched sibling HSCT (MST) with respect to graft source

Graft source	Engraftment	TRM	Recurrence	GVHD
BM (N = 3)	2/3 (66.66 %)	1/3 (33 %) due to GVHD	0/2 (0 %)	2[1 Acute, 1 Chronic] (66.66 %)
PBSC (N = 14)	14/14 (100 %)	2/14 (14 %) –1 due to infection –1 due to hematuria	1/14 (7 %)	4 [4 Chronic] (28.5 %)

The table shows the graft specific results of matched sibling transplantation (MST)

Table 7 The incidence of GVHD with graft source and conditioning regimens

	GVHD correlation with respect to graft source and conditioning regimen
Graft source	
BM (N = 3)	2[1 Acute, 1 Chronic] (66.66 %)
PBSC (N = 14)	4 [4 Chronic] (28.5 %)
Conditioning regimen	
BCA (N = 15)	1 Acute, 4 chronic
TCA (N = 2)	1 Chronic

Umbilical cord transplantation: Nine children with beta-thalassemia major were undergone partially HLA-matched unrelated UCT. A second transplant with 2 unrelated cord

blood units was attempted in two patients who had primary graft failure. Five of the 9 (56 %) patients engrafted promptly with 50–100 % donor chimerism. They engrafted at a median of 17 days (range 12–19). One patient is transfusion free for 48 months; a second patient is transfusion free for 30 months and a third is transfusion free for 21 months. There was no TRM. Four of the 9 (44 %) children had autologous recovery without engraftment. Primary graft rejection is the major complication. Post transplant complications were mild hepatic veno-occlusive disease (N = 1), acute GVHD grade II (N = 2) and grade I (N = 1), CMV interstitial pneumonia (N = 1), asymptomatic CMV reactivation (N = 7) and 1 chronic GVHD. UCB has the advantages of rapid availability and low risk of severe GVHD despite donor–recipient HLA disparity. We demonstrated the feasibility of this procedure in the setting of a developing country.

Table 8 Cost comparison of thalassemia treatment

Cost of supportive therapy of thalassemia	Matched sibling HSCT	Unrelated cord HSCT
Blood transfusion		
Chelation		
Investigation		
Miscellaneous expenditure		
Total cost		
	Rs 2,00,000/year for life time	Rs 6,00,000 (One time cost)
		Rs 20,00,000 (One time cost)

The table shows cost effectiveness of transplantation in thalassemia treatment

Discussion

Incidence of thalassemia: Incidence of beta-thalassemic trait ranges between 8 and 15 %. There are 65,000–67,000 β -thalassemia patients in India. Every year 9000–10,000 cases being added. The carrier rate for β -thalassemia gene varies from 1 to 3 % in Southern India to 3 to 15 % in Northern India. Sindhis and Punjabis from Northern India, Bhanushali's, Kutchis, Lohana's from Gujarat, Mahar's, Neobuddhist's, Koli's and Agri's from Maharashtra, & Gowda's and Lingayat's from Karnataka etc. have a higher carrier rate [1, 2].

Prevalence of thalassemia: Overall prevalence of β -thalassemia trait is 2.78 % and it varies from 1.48 to 3.64 % in different states of India. Sixty to 80 million people in the world carry the beta thalassemia trait alone. β -thalassemia is the most common single gene disorder in India. It is a huge public health problem worldwide. Recently there has been increase in survival of these patients. Therefore the prevalence is also increasing. There are 240 million carriers of β -thalassemia worldwide. In India it accounts up to 30 million (mean prevalence of 3.3 %.) Punjabis, Sindhis, Bengalis, Jains and Muslims. With modern supportive therapy, 68 % of patients with beta-thalassemia are alive at the age of 35 [1, 2].

Transfusion and Chelation Versus HSCT

Transfusion and Chelation: This gives almost normal life if compliant with Treatment. It is required life long and is expensive. Also there is risk of cardiac disease, growth failure, hypogonadism, diabetes and liver disease. Annual cost of transfusion and chelation is approximately Rs. 2,00,000 [3].

HSCT: It has its immediate risk of TRM but later it gives good quality of life. It has one time cost and less

expensive than life long transfusion. It has also the issues of GVHD, infections, interstitial Pneumonia and regimen related toxicity. The cost of MST in 30 kg child is approximately Rs. 6 lakh while that of UCT is 20 lakh [3].

Also our data is comparable with international outcomes and that with other Indian reports (Table 5). The disease free interval is excellent in low risk groups while it decreases with higher risk [4–7].

Table 6 shows the comparison of outcome of sibling HSCT with respect to graft source. Though the representative sample size is less for comparison, it shows that PBSC source is acceptable with such a good outcome in comparison to BM source (100 % engraftment in PBSC versus 66.66 % in BM and 28.5 % chance of GVHD in PBSC versus 66.66 % in BM).

Upper curves of Fig. 1 shows the overall survival (OS) of MST patients is 83 % while OS of UCT patients is 89 %. Lower curves show that TRM of MST is 17.64 % and TRM of UCT is 0 %.

Figure 2 shows that thalassemia free survival MST patients was 70 % while that of UCT patients was 33 %.

Figure 3 shows the thalassemia free survival of MST patients according to risk classes being 100 % for class 1, 86 % for class 2, 50 % for class 3. This emphasizes early transplantation in lower risk cases increases the chances of thalassemia free survival.

Figure 4 shows the correlation of age, hepatomegaly and thalassemia free survival (%) in MST. The upper curve with age less than 7 years and without hepatomegaly has best thalassemia free survival (100 %) and the thalassemia free survival decreases with increasing age (80 %) and hepatomegaly (75 %). Patients with age ≥ 7 years and hepatomegaly have thalassemia free survival of only 50 %.

Figure 5 shows the TRM (%) in MST according to cell dose ($\times 10^8$ MNC/kg). It suggests that TRM was high (12.5 %) for stem cell dose $\leq 2.4 \times 10^8$ MNC/kg. It decreased further to 6.25 % when cell dose was $> 2.4 \times 10^8$

Fig. 1 Overall survival (OS) and transplant related mortality (TRM) of MST and UCT

Fig. 2 Thalassemia free survival according to graft source

Fig. 3 Thalassemia free survival of MST patients according to risk classes

Fig. 4 correlation of age, hepatomegaly and thalassemia free survival (%) in MST

Fig. 5 TRM (%) according to cell dose ($\times 10^8$ MNC/kg)

MNC/kg but $\leq \times 10^8$ MNC/kg. TRM was 0 % when cell dose was $>5 \times 10^8$ MNC/kg. Thus MNC dose is predictor of transplant outcome.

Figure 6 shows the thalassemia free survival according to CD-34 in cell dose. ($\times 10^6$ /kg). When CD 34 was $>2.7 \times 10^6$ /kg, thalassemia free survival was 80 %

and it decreased to 50 % when CD-34 was $<2.7 \times 10^6$ /kg. Thus CD-34 may also be predictor of transplant outcome.

Most of our patients were moderate to high risk, cachexic and from low socioeconomic condition. When this fact is considered the present results seem to be still more encouraging.

Fig. 6 Thalassemia free survival according to CD 34 in cell dose ($\times 10^6/\text{kg}$)

Umbilical cord hematopoietic stem cells are increasingly used as an alternative to bone marrow; advantages include ready availability, no risk to the donor, low rate of viral contamination and low risk of GVHD. Disadvantages include low stem-cell dose for larger patients and lack of stem cells for boost infusions following the initial procedure.

Conclusion

- (1) HSCT is feasible than conservative management for thalassemia.
- (2) HSCT has become now the accessible and affordable for thalassemia in India.
- (3) Physicians should refer the patient earliest for HSCT.

Funding There was no financial support for this publication.

Compliance with Ethical Standards

Conflict of Interest Authors declare no conflict of interest.

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