

## **Allogeneic Bone Marrow Transplantation in b-Thalassaemia - Single Centre Study**

K. U. Hashmi ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 B. Khan ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 P. Ahmed ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 E. Hanif ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 M. Naeem ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 H. Iqbal ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 H. S. Malik ( Armed Forces Bone Marrow Transplant Centre, Rawalpindi. )  
 I. Hussain ( Armed Forces Combined Military Hospital\*, Rawalpindi. )  
 S. Rasul ( Armed Forces Combined Military Hospital\*, Rawalpindi. )

### **Introduction**

The term Thalassaemia refers to various types of hereditary anaemias, which are identified by a reduced production of one of the globin chains, which forms the haemoglobin molecule. In b-Thalassaemia there is deficient or absent synthesis of b-globin chains that constitute the adult haemoglobin molecule, which causes several deleterious effects on erythrocyte production and survival. Hemolysis and ineffective erythropoiesis leads to chronic anemia with erythroid marrow hyperplasia.<sup>1</sup>

Thalassaemias are widely distributed in the Mediterranean, Middle Eastern and Asian countries and occur with significant incidence world wide in populations that originate from these areas. Thalassaemia probably represents the most common single gene disorder to cause a major public health problem in the world. In the Mediterranean and Arab Gulf areas there are more than 200,000 homozygous b-Thalassaemia patients. In certain areas such as Greece, Mediterranean littoral of Italy, Iran, Southern Russia, India and South Asia, where 10-15% of population carry the b-Thalassaemia gene, the homozygous birth rate is between 1:150 and 1:200.<sup>2</sup>

b-Thalassaemia is a major health problem in Pakistan. It is the most common genetic disorder in the country with a gene frequency of 5-8%<sup>3,4</sup> and approximately 5000 children are diagnosed each year in all ethnic groups. Transfusion and regular iron chelation with desferrioxamine remains the cornerstone of the conventional treatment for severe b-Thalassaemia. However the lack of availability of proper medical treatment and frequent failure of patients to comply with prescribed therapy remains unresolved problems.<sup>5-</sup>

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Though regular iron chelation slows down progressive iron overload, however iron overload keeps on increasing gradually which may further be enhanced with the concomitant presence of chronic active hepatitis. Since b-Thalassaemia is a genetic disease in which defect lies in the haemopoietic marrow, therefore it is rational to eliminate the disordered marrow and substitute it with allogeneic marrow able to produce normal haemoglobin. The only radical cure of thalassaemia is to correct the genetic defect by allogeneic bone marrow transplantation.<sup>8,9</sup> First time in 1982; a 14 months-old untransfused thalassaemic patient was transplanted in Seattle from his HLA identical sister. At the same time a 14 years old thalassaemic patient who had received 150 red cell transfusions was transplanted in Pesaro.<sup>10,11</sup> The initial results of BMT were disappointing. Conditioning regimens used in these patients included high doses of cyclophosphamide and Total Body Irradiation (TBI). A high percentage of graft failure and early toxicity was observed. From 1983 conditioning regimens were modified which included combination of busulphan and cyclophosphamide. Several BMT teams have ongoing clinical experience. The results of marrow transplantation have improved steadily with improved conditioning regimens and major progress in the management of transplant related complications. This is due to the use of cyclosporine, more effective treatment for CMV infection, improvement of aseptic techniques and evolution of systemic antibiotic therapy.<sup>12</sup>

We describe our experience in the cure of thalassaemia by bone marrow transplantation from HLA identical siblings.

## Patients and Methods

Between August 2001 and November 2003, nineteen patients, between the ages of 1-14 years (median age 5 years) were transplanted for the treatment of homozygous  $\beta$ -Thalassaemia. Recipient and donors were genotypically HLA identical. All recipients/donors pairs, except one, were ABO identical. Disease status was categorized into three classes (class I, II and III) according to modified risk criteria derived from Pesaro Risk Group classification.<sup>13,14</sup> These risk factors were defined as hepatomegaly  $>2\text{cm}$ , portal fibrosis  $>\text{grade I}$  (Knodell scoring), and inadequate compliance with chelation therapy.<sup>15</sup> According to our designed criteria adequate iron chelation was defined as chelation with desferrioxamine initiated within 18 months of the first transfusion and administered subcutaneously for 8-10 hours at least five days each week. Inadequate compliance was failure to maintain adequate compliance with raised serum ferritin levels ( $>200\text{ng/ml}$ ) and this categorization could not be corrected by subsequent intensive chelation. On the basis of these three risk factors and classes, eligibility criteria for allogeneic BMT were established (Table 1). Class-I patients had none of these risk factors, Class-II patients had one or two risk factors and Class-III patients had all the three risk factors.<sup>16</sup> (Table 1)

Those thalassaemic patients who were infected with HCV were further categorised in group A and group B. On the basis of these risk factors and classes, the eligibility criteria for Hepatitis C negative and positive thalassaemic patients were designed (Table 2). Patients infected with Hepatitis C virus falling in group A were eligible to undergo BMT and patients in group B were not eligible for BMT.

Class I and II patients received conditioning<sup>17-19</sup> with Busulphan ( $3.5\text{ mg/kg/day}$ ) from day 9 till day 6 for eradication of disordered haemopoietic marrow and cyclophosphamide ( $50\text{mg/kg/day}$ ) from day 5 till day 2 for the suppression of immune system (Bu 14/Cy 200).

Class III patients received long duration conditioning protocol<sup>20</sup> consisting of hydroxyurea  $30\text{mg/kg}$  (from day 45 till day 11), azathioprine  $3\text{mg/kg}$  (from day 45 till day 11), fludarabine  $20\text{mg/kg}$  (from day 17 till day 11), followed by standard Bu14/Cy200, as used for class I and II thalassaemic patients. Seventeen patients received bone marrow harvest from young sibling donors; whereas two patients received Peripheral Blood Stem Cell (PBSC) harvest from their adolescent

sibling donors and these donors received G-CSF  $5\text{mg/kg}$  for 5 days prior to PBSC harvest. Peripheral blood stem cells were harvested on day 2 and day 1 using Cobe-spectra cell separator. Central venous access line was passed for stem cell harvest from these donors. Median apheresis time was 230 minutes (200 - 250 minutes), depending upon the volume and rate of blood flow in the apheresis system. The standard dose of mononuclear cells (MNC  $>4.0 \times 10^8/\text{kg}$  body wt of the recipient) was achieved by apheresis system.

Bone marrow harvest/PBSC harvest was infused on day 0 of the conditioning under cover of steroids and antihistamines. GvHD was diagnosed and graded both clinically and histologically.<sup>21</sup> As prophylaxis against GvHD, cyclosporin ( $5\text{mg/kg/day}$  in two divided doses) and prednisolone ( $0.5\text{mg/kg/day}$  started from day 2 onwards) were given. The IV dose of cyclosporin was reduced to  $3\text{mg/kg/day}$  from day +6 and then switched over to oral cyclosporin therapy as the patient's condition permitted. The oral dose of the cyclosporin at the time of switch over from I/V dose was doubled. Cyclosporin dose was adjusted according to drug therapeutic levels as well as according to renal status of the patient. Trough levels of cyclosporin were maintained in between 200 and 300 ng/ml and continued for 06 months, then gradually tapered off in next six months (Total duration one year). Prednisolone was gradually tapered off in 90 days. In class III patients short I/V methotrexate ( $10\text{mg/m}^2$ ) was given on day 1, 3, 6 and 11 along with folinic acid rescue therapy.

All Thalassaemia patients also received IV immunoglobulin  $500\text{mg/kg}$  on day 1 and then  $250\text{mg/kg}$  on day 8 and day 22. All patients received G-CSF  $5\text{mg/kg/day}$  from day 5 till the neutrophil recovery ( $\text{ANC} >0.5 \times 10^9/\text{l}$ ).

Anti microbial therapy consisting of broad-spectrum antibiotics, antivirals and antifungals in prophylactic doses started from day 2 (conditioning regimen related neutropenic phase) and switched over to therapeutic doses according to the clinical status of the patient (neutropenic fever) as per laid down guidelines.<sup>22</sup> All patients were nursed in special rooms equipped with positive pressure filtered (Hepa filters) air-conditioning system. During early post transplant aplastic phase, the patients were given leuco-depleted, irradiated blood products. Early haematological recovery was defined as absolute neutrophil count of  $>0.5 \times 10^9/\text{l}$  and platelet count of  $>20 \times 10^9/\text{l}$ . GvHD was diagnosed and graded both clinically and histologically.<sup>13</sup>

## Results

Out of 19 patients who were transplanted, 14 were

males and 5 females (male: female ratio 2.8:1). Age ranged from 1 to 14 years (median 5 years). Nine patients (47.3%) fulfilled criteria for class I, whereas 7 (36.8%) were placed in class II. Three patients (15.8%) were in class III.

The average number of transfusions before transplant was 59.7 (range 3 - 177), 4 patients were Anti-HCV positive but HCV RNA was negative. All patients were negative for Hepatitis B and HIV. As per laid down criteria for allogeneic BMT, pre-transplant classification was based on Knodell scoring (histological hepatitis activity index with hepatic fibrosis), which ranged from 0/22 - 9/22 (average 5.6/22) and serum ferritin levels, which ranged from 1000 - 3610 ng/ml (average 1935.7 ng/ml) in these patients. Based on the same risk group classification criteria, the average liver size ranged from 0 - 6 cm (average 2.16cm) above the normal hepatic span.

Uptil now all these patients have been followed up for 27 months. Engraftment was achieved in all class I patients (n=9), where as in class II (n=7) and class III (n=3), graft rejection was observed in one patient from each class. Median time to achieve absolute neutrophil recovery of  $0.5 \times 10^9/l$  was 13 days (Range 12-17 days), platelets count  $>20 \times 10^9/l$  was 15 days (range 11-32 days) and reticulocyte count  $>0.5\%$  was 15 days (range 10-20 days).

Major post transplant non-infective complication encountered was acute GvHD. GvHD skin grade I occurred in 79.0% (n=15), GvHD skin grade II 10.5% (n=2), GvHD intestine grade II 10.5% (n=2), GvHD skin grade III 15.7% (n=3), GvHD liver grade-III 10.5% (n=2), GvHD skin and liver grade-IV 5.2% (n=1). Chronic GvHD of Skin and liver was observed in one patient (5.2%). Other non-infective complications were Veno-Occlusive Disease (VOD), hypertension 42.1%(n=8), generalized fits 10.5%(n=2) and haemorrhagic cystitis 15.7% (n=3). Grade I and grade II GvHD was effectively managed by escalating the dose of cyclosporin and steroids, whereas in class-III and class IV GvHD, high dose steroid pulse therapy as well as interleukin II receptor antibodies were used beside cyclosporin to control GvHD. Three patient developed cyclosporin induced nephrotoxicity and cyclosporin was replaced with mycophenolate mofetil.

Post-transplant infective complications were pseudomonas septicaemia 10.5% (n=2), CMV infection 5.2%(n=1), fungal pneumonia 5.2% (n=1) and tuberculosis 5.2% (n=1).

Of the 19 patients who were transplanted, 13 have fully recovered with 68.4% disease free survival. The main causes of mortality were VOD liver and

GvHD 5.2% (n=1), septicemia 5.2% (n=1), fungal pneumonia 5.2% (n=1) and Table 1. Risk factors and pre-transplant classification.

A. Pesaro group risk factors	
1. Hepatomegaly	
2. Portal fibrosis on liver biopsy	
3. Inadequate iron chelation	
B. Modified risk factors	
1. Hepatomegaly (2 cm above normal span)	
2. Knodell scoring	
a) Portal fibrosis (0-4)	>9/22
b) Hepatitis activity index (HAI) (0-18)	>14
1) Periportal necrosis (0-10)	>8/18
2) Intralobular degeneration and focal necrosis (0-4)	
3) Portal inflammation (0-4)	
3. Inadequate chelation	
Serum ferritin	>2000ng/ml
C. Classification	
Class I	All risk factors absent
Class II	One or two of the three risk factors present
Class III	All risk factors present

Table 2. Eligibility criteria. For HCV negative patients

1. Patients in Pesaro risk class I, II and III
2. Age upto 14 years
3. HLA matched sibling donor
4. Overall HAI <9/22

For HCV positive patients

Group A:  
HCV antibody positive  
HCV PR positive/negative  
HAI 0-8  
Fibrosis 0-1

Group B:  
HCV antibody positive  
HCV PCR positive  
HAI >8  
Fibrosis >1

Group A patients eligible and group B patients not eligible for BMT.

tuberculosis 5.2% (n=1).

Engraftment was achieved in all Class I patients (n=9), whereas in Class II (n=7) and Class III (n=3), graft rejection was observed in three patients, two from class II and one patient from class III. However one of the patients

Table 3. Outcome.

	Class I (n=9)	Class II (n=7)	Class III (n=3)	Overall (n=15)	Disease free survival (DFS)
Survival	77.7% (n=7)	71.4% (n=5)	33.3% (n=1)	78.9% (n=15)	
Mortality	22.2% (n=2)	14.3% (n=1)	33.3% (n=1)	21.0% (n=4)	68.4% (n=13)
Rejection	-	14.3% (n=1)	33.3% (n=1)	10.5% (n=2)	

from class II received a second graft from the same sibling and achieved complete engraftment. So, initially three patients rejected the allograft. Thus over all rejection was 10.5% (n=2) (Table 3).

## Discussion

Though prognosis of patients with transfusion dependant homozygous b-Thalassaemia has been improved with regular blood transfusion and iron chelation therapy. The need for definite information about long-term prognosis of patients with b-Thalassaemia major has increased since allogeneic bone marrow transplantation emerged as an alternative treatment.<sup>23</sup>

BMT has been established as a therapeutic option in b-Thalassaemia major. The centre with the largest experience and the most impressive data is that of Lucarelli and his colleagues at Pesaro, Italy. Previously published data from this centre shows 97% overall survival with 94% event free survival in Class I patients and overall survival in Class II patients was 89% with 85% disease free survival. Whereas in class III over all survival was 76% with 70% disease free survival.<sup>24,25</sup> However the recently published 14 years data from the same centre indicates 91% thalassaemia free survival in class I (n=124) and 84% thalassaemia free survival in class II (n=297), where as 58% (n=122)

thalassaemia free survival in class-III patients and 62% disease free survival in adult thalassaemia patients (n=109). Overall survival including all groups represents 73% thalassaemia free survival. Since April 1997 a new preparatory regimen for Class III thalassaemic patients namely protocol 26 has been elaborated with the aim to increase bone marrow eradication and immunosuppression by adding hydroxyurea, azathioprine, and fludarabine to the Bu14/Cy160. Uptil now 23 class-III patients were transplanted through this protocol and preliminary results are consistent with 96% thalassaemia free survival.<sup>8</sup>

The post transplant data published from two British Centres (Westminster and Manchester) in thalassaemic patients show 71.0% over all survival.<sup>15</sup> Similarly data recently published from Bismillah Taque Blood Disease Centre Karachi, Pakistan, also show 66.6% post transplant survival in b-Thalassaemia patient.<sup>5</sup>

Since the establishment of Armed Forces Bone Marrow Transplant Centre Rawalpindi, Pakistan a total of 19 b-Thalassaemia patients have been transplanted from HLA identical siblings. Out of these, 13 have fully recovered with 68.4% disease free survival whereas overall survival is 78.9% (n=15). Disease free survival in class I was 77.7% (n=7) whereas in class II it was 71.4% (n=5). 31.5% patients (n=6) developed post transplant infectious complications. Three patients died of pseudomonas septicemia, fungal pneumonia and tuberculosis. The infection-related mortality was 15.75%. The mortality due to GvHD was minimal 5.7% (n=1). Three patients rejected allogeneic graft. Of these three patients, one achieved successful reconstitution after second transplant. The overall rejection was 10.5% (n=2). Graft rejection was observed in class II and Class III patients only.

Our results are comparable with the above mentioned centres, however the relatively lower survival in our patients as compared to Pesaro group are due to irregular pre transplant chelation therapy and poor post transplant compliance.

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## **Abstract**

**Objective:** To evaluate outcome of allogeneic BMT in b-Thalassaemia at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan

from August 2001 to November 2003.

**Methods:** Nineteen patients with b-Thalassaemia underwent allogeneic BMT/PBSC transplantation from HLA identical sibling donors. Patients were classified in three groups according to Pesaro (Italy) risk classification. Class-I(n=9) and Class-II(n=7) patients received conditioning with busulphan/cyclophosphamide, whereas Class-III(n=3) patient received conditioning with hydroxyurea, azathioprine, fludarabine, alongwith Bu14/Cy 200. Cyclosporine, prednisolone and methotrexate were given for GvHD prophylaxis. Stem cells dose infused was  $>4.0 \times 10^8$ /kg body weight of the patient.

**Results:** Engraftment was achieved in all Class-I patients, whereas in Class-II and Class-III, graft rejection was observed in one patient from each class. Median time to achieve absolute neutrophil recovery ( $> 0.5 \times 10^9/l$ ) was 13 days, platelet count ( $>20 \times 10^9/l$ ) was 15 days and reticulocyte count ( $> 0.5\%$ ) was 15 days. Acute GvHD was observed in 15 patients. One patient developed grade IV GvHD (liver and skin) and died within 30 days post BMT. Post transplant infectious complications were pseudomonas septicemia, disseminated fungal infection, CMV pneumonia and tuberculosis. Three patients died of these complications during post transplant period (31-90 days). Median stay in hospital was 25 days.

**Conclusion:** Allogeneic BMT is the only curative therapy for b-Thalassaemia patients, however the success rate can be increased if the patients are selected carefully and transplanted at an early age (JPMA 54:499;2004).