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Bone Marrow Transplantation in Thalassemia Major Patients Using "Short" Anti-Thymocyte Globulin Therapy in Shiraz, Southern Iran

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ABSTRACT

Allogeneic bone marrow transplantation (BMT) was performed on 113 Iranian transfusion-dependent thalassemia major patients from May 1993 through September 2003. To have at least 2 years follow-up, we report BMT on 90 patients transplanted up to December 2001. The donors were human leukocyte antigen (HLA)-identical, mixed lymphocyte culture (MLC)-nonreactive siblings ($n = 74$) on parents ($n = 6$); HLA-identical MLC-reactive siblings ($n = 5$) or parents ($n = 1$); and one HLA antigen-mismatched sibling ($n = 4$). The induction regimen in 11 patients was oral busulfan (BU) (14 mg/kg) and IV cyclophosphamide (CY; 200 mg/kg); in fifteen patients it was BU (15 mg/kg) and cyclophosphamide (CY; 200 mg/kg); in 47 patients, BU (15 mg/kg), CY (200 mg/kg), and short course of anti-thymocyte globulin (ATG, horse; 40 mg/kg including 10 mg/kg on days -2, -1, $+1$, $+2$); and in 15 patients, BU (15 mg/kg) CY (200 mg/kg), and ATG (60 to 100 mg/kg; 10 mg/kg at 3 to 5 days before and after BMT). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and prednisolone. The group who received BU (14 mg/kg) and CY (200 mg/kg), as compared to the group receiving BU (15 mg/kg) and CY (200 mg/kg), was of younger age and lower risk; median age 7 versus 10 years, and 46% versus 7% in Lucarelli's risk group class I (the best prognostic group), respectively. These patients showed a lower disease-free survival (DFS), namely 64% versus 73%, with a follow up of 2 to 10.5 years. Thus from 9.5 years ago, our standard protocol for BU has been 15 mg/kg. The group who received "short" ATG (40 mg/kg), BU (15 mg/kg), and CY (200 mg/kg) showed almost the same outcome as the group who received a higher dose of ATG (60 to 100 mg/kg), namely DFS 72% versus 73%, respectively, despite the fact that half of both groups were included in the Lucarelli's risk group class III (the worst prognostic group) 49% versus 53%.

We showed the same DFS for the patients who received BU (15 mg/kg), CY (200 mg/kg), and no ATG compared with the ATG group (73% vs 72%), but 27% of the group without ATG developed grade IV acute GVHD and 54% developed chronic GVHD. In the group with short ATG, 15% and 17% of patients developed grade IV acute and chronic GVHD, respectively. There was no significant difference for falls in platelets and white blood cell or engraftment days and the number of packed red blood cell transfusions among the groups. The median hospital stay was longer for the group with BU (15 mg/kg), CY (200 mg/kg) namely 81 versus 61 to 65 days. Second bone marrow infusions were needed in 6% and 20% of patients who received ATG doses of (40 versus 60 to 100 mg/kg; respectively (1 to 2 month post-BMT). BU at a dose of 15 mg/kg was more effective than 14 mg/kg BU for its myeloablative properties. By adding "short" ATG course to the conditioning regimen, the incidence of grade IV acute and chronic GVHD was reduced in thalassemic patients, especially when an HLA disparity was present.

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Table 1. The Pretransplant Characteristics in Patients With Thalassemia Major Based on the Different Conditioning Regimens in Shiraz (1993–2003)

	Groups				
	A1	A2	B1	B2	Total
Patients	*n* = 11	*n* = 15	*n* = 47	*n* = 15	*n* = 88
Age, range (median), year	2–11 (7)	5–20 (10)	5.5–17.5 (11)	5–16 (12)	2–20 (10.5)
Disease stage, Lucarelli risk group* *n* (%)					
Class I	5 (46)	1 (7)	4 (9)	0	10 (11)
Class II	2 (18)	8 (53)	20 (42)	7 (47)	37 (42)
Class III	4 (36)	6 (40)	23 (49)	8 (53)	41 (47)
Male:female (*n*)	7:4	9:6	25:22	9:6	50:38
Sex mismatched, *n* (%)	6 (55)	6 (40)	25 (53)	6 (40)	43 (49)
Major blood group mismatched, *n* (%)	0	4 (27)	7 (15)	3 (20)	14 (16)
Minor blood group mismatched, *n* (%)	2 (18)	4 (27)	6 (13)	2 (13)	14 (16)
Donor-patient histocompatibility, *n* (%)					
HLA-identical MLC nonreactive					
Sibling	11 (100)	13 (87)	39 (83)	10 (67)	73 (83)
Parent	—	—	5 (11)	1 (7)	6 (7)
HLA-identical MLC reactive					
Sibling	—	1 (7)	1 (2)	2 (13)	4 (4.5)
Parent	—	—	1 (2)	—	1 (1)
One HLA antigen mismatched					
Sibling	—	1 (7)	1 (2)	2 (13)	4 (4.5)
Total siblings	11 (100)	15 (100)	41 (87)	14 (93)	81 (92)
Total parents	—	—	6 (13)	1 (7)	7 (8)

*Class I, no hepatomegaly, no portal fibrosclerosis, and adequate chelation therapy; class II, with hepatomegaly, >2 cm palpable, or portal fibrosclerosis and/or inadequate chelation therapy; class III, with hepatomegaly, portal fibrosclerosis, and inadequate chelation therapy.

TRANSPLANTATION OF hematopoietic stem cell (HSCT) is today an established therapy for a variety of congenital or acquired severe disorders of the hematopoietic system as well as for chemo- or radiosensitive malignancies. In the last decade with better definition of the human leukocyte antigen (HLA) system and the immunology of transplantation, improved clinical results have led to use of HSCT at earlier stages of the natural history of several diseases.

Having 20,000 transfusion-dependent beta-thalassemia patients in Iran, 2500 of whom are living in Fars province in the South of Iran where 7% of the population are carriers of beta-thalassemia gene, we established allogeneic HSCT as a curative treatment for these patients and for chemosensitive malignancies. In this article report we described the various conditiong regimens and patient outcomes.

PATIENTS AND METHODS

From May 1993 to December 2001, 90 patients with transfusion-dependent beta-thalassemia major underwent allogeneic bone marrow transplantation (BMT) from donors who were HLA-identical, mixed lymphocyte culture (MLC)- nonreactive siblings (*n* = 74) or parents (*n* = 6); from HLA-identical MLC-reactive siblings (*n* = 5) or parents (*n* = 1); and from one HLA-antigen mismatched sibling (*n* = 4); (Table 1). The induction regimen in 11 patients was oral busulfan (BU; 14 mg/kg) and intravenous cyclophosphamide (CY; 200 mg/kg; group A1). In 15 patients it was BU (15 mg/kg) and CY (200 mg/kg; group A2). In 47 patients it was BU (15 mg/kg), CY (200 mg/kg), and a short course of horse antithymocyte globulin (ATG; 40 mg/kg; 10 mg/kg on days −2, −1, +1, +2; group B1). In 15 patients it was BU (15 mg/kg), CY (200 mg/kg), and ATG (60 to 100 mg/kg; 10 mg/kg, 3 to 5 days before and after BMT; group B2). Graft-versus-host disease (GVHD) prophylaxis used intravenous cyclosporine 5 mg/kg from day −2 decreased to 3 mg/kg intravenously intravenously or 12.5 mg/kg orally on day +6 after marrow transplantation) which was tapered off at 18 to 24 months post-BMT (−10% every 1 month). In addition methylprednisolone (0.5 mg/kg intravenously) was prescribed from day −1 and continued until day +21 post-BMT.

In all the patients, liver biopsies were performed before transplantation to evaluate the degree of iron overload and determine the patient class according to Lucarelli's classification: class I (no hepatomegaly, no portal fibrosclerosis, and adequate chelation therapy), class II (hepatomegaly, >2 cm palpable, or portal fibrosclerosis and/or inadequate chelation therapy), class III (hepatomegaly, portal fibrosclerosis, and inadequate chelation therapy).

The patients were managed in single-bed positive-pressure HEPA-filtered rooms.

Marrow was collected (up to 20 mL/kg) from the donors under general anesthesia in the operating room. Bone Marrow was aspirated from the posterior and right anterior iliac crests, then infused via a peripheral intravenous line.

Prophylactic antibiotics and antifungals were administered to all patients; intravenous immunoglobulin and acyclovir were given to patients as prophylaxis for viral infection. All cellular blood components were irradiated with gamma rays (3000 cGy) to prevent transfusion-associated GVHD.

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Table 2. Transplant and Post-transplant Data in Patients With Thalassemia Major Based on the Different Conditioning Regimens in Shiraz (1993–2003)

	Groups			
	A1	A2	B1	B2
Conditioning regimens (mg/kg)				
BU	14	15	15	15
CY	200	200	200	200
ATG			40	60–100
Bone marrow volume, range (median), mL	200–680 (400)	350–1250 (800)	330–1200 (650)	400–1240 (800)
Total nucleated cells of donor marrow/kg wt of recipient, range (median)	4–10 (5) $\times 10^8$	1.8–8.7 (3.3) $\times 10^8$	2–7 (4.4) $\times 10^8$	2.3–7 (3.5) $\times 10^8$
Day of plt drop to 00,000/mm^3, range (median)	0–+8 (+4)	+1–+6 (+4)	−3–+6 (+4)	−1–+8 (+3)
Day of WBC drop to <1000/mm^3, range (median)	0–+7 (+3)	−2–+6 (+2)	−2–+5 (+2)	0–+6 (+1)
Day of plt engraftment (>30,000/mm^3), range (median)	+16–+49 (+23)	+13–+74 (+23)	+5–+125 (+20)	+9–+64 (+25)
Day of PMN engraftment (>500/mm^3), range (medium)	+12–+33 (+15)	+11–+28 (+14)	+10–+92 (+19)	+11–+59 (+15)
Second BM infusion	1 (9%)	—	3 (6%)	3 (20%)
n (%)	98		34,35,56	41,49,77
days, post-BMT				
No. of plt concentrate transfusions, range (median)	3–26 (6)	5–17 (8)	2–34 (10)	2–26 (11)
No. of RBC transfusions, range (median)	2–9 (3)	1–10 (5)	0–13 (4)	0–13 (5)
Hospital stay, range (median), days	52–130 (65)	50–133 (81)	44–175 (61)	52–119 (65)
Acute GVHD, *n* (%)				
Grade I	6 (55)	6 (40)	18 (38)	5 (33)
Grade II	2 (18)	2 (13)	7 (15)	3 (20)
Grade III	1 (9)	1 (7)	5 (11)	—
Grade IV	1 (9)	4 (27)	7 (15)	3 (20)
Total	10 (91)	13 (87)	37 (79)	11 (73)
Chronic GVHD				
Limited	3 (27)	4 (27)	5 (11)	—
Extensive	—	4 (27)	3 (6)	—
Total	3 (27)	8 (54)	8 (17)	—

Abbreviations: BU, busulfan; CY, cyclophosphamide; ATG, anti-thymocyte globulin, Lymphoglobuline Merieux. (Pasteur Merieux, Lyon, France); plts, platelets; WBC, white blood cells; PMN, neutrophils; RBC, packed red blood cells; GVHD, graft-versus-host disease.

For major ABO-mismatched transplants, we performed gravity sedimentation with hydroxyethyl starch (HES) at a ratio of 1:8 HES to marrow volume for about 2 hours.

In the case of ABO incompatibility; for the first 2 weeks following transplantation, transfusion support consisted of the administration of recipient type or O type red blood cells or plateletes. After 2 weeks donor type transfusions were used

Therapy of GVHD was undertaken with methylprednisolone (1 to 10 mg/kg intravenously ± ATG.) Therapy of suspicious or proven CMV infection used ganciclovir and intravenous immunoglobulin. From day +35 to +180, all patients received prophylaxis for pneumocystis carini with co-trimaxazols (5 mg/kg/d × 2 times a week).

RESULTS

The first 11 patients who received conditioning therapy with BU (14 mg/kg) and CY (200 mg/kg) included two deaths and three cases of mixed chimerism. A 9.5-year old male patient received BU (14 mg/kg), CY (200 mg/kg), and ATG (15 mg/kg/d on days −3 to −1) and expired 7 months post-BMT due to obstructive airway disease (bronchiolitis obliterans) associated with extensive chronic GVHD. The other case, a 15-year-old boy, received BU (15 mg/kg) ATG, (100 mg/kg on days −5 to +5), and CY (130 mg/kg) rejected the donor marrow 3 months post BMT. From October 1994 (16 months later) we increased the dose of BU to 15 mg/kg, and from January 1995 (3 months later) added ATG (10 mg/kg) on days −2, −1, +1, +2 or days −5 to +5 (Table 2).

There was no significant difference in relation to need for blood component support between the four groups, but the hospital stay was longer for the group who received only BU (15 mg/kg) and CY (200 mg/kg), since 34% developed grade III or IV acute GVHD. In contrast the ATG group only 20% to 26% showed these complications. No chronic GVHD was observed among patients who received ATG (60 to 100 mg/kg) despite HLA age disparity. A lower incidence was noted among the group who received 40 mg/kg ATG, compared to those not receiving ATG. A second bone marrow infusion was needed in the group receiving ATG (6% to 20%).

Table 3. Outcome of Bone Marrow Transplant in-Patients With Thalassemia Major Based on the Different Conditioning Regimens in Shiraz (1993–2003)

	Group				
	A1 (n = 11)	A2 (n = 15)	B1 (n = 47)	B2 (n = 15)	Total (n = 88)
Follow-up, range (median), year	9–10.5 (9.5)	2–9 (7)	2–9 (6.5)	4–7 (5.5)	2–10.5 (6)
Death					
n (%)	2 (18%)	1 (7%)	9 (19%)	3 (20%)	15 (17)*
day, post-BMT	22, 365	180	13–570	12, 46, 64	P = .7432
Mixed chimersim					
n (%)	3 (27%)	1 (7%)	3 (6%)	1 (7%)	8 (9)
day, post-BMT	150, 240, 250	60	56, 90, 90	120	
Stable, *n*	2	1	3	1	7 (8%)
Recurrence of thalassemia					
n (%)	2 (18)	3 (20%)	4 (9)	1 (7%)	9 (10)**
day, post-BMT	190	90, 150, 600	60, 90, 365, 700	90	
Full donor chimerism, n (%)	5 (54)	10 (67)	31 (66)	10 (67)	57 (64)
Disease-free survival, *n* (%)	7 (64)	11 (73)	34 (72)	11 (73)	64 (72)
Overall survival, *n* (%)	9 (82)	14 (93)	38 (81)	12 (80)	73 (83)
					P = .5527

*Class I = 0, class II = 7, class III = 8.
**Class I = I, class II = 3, class III = 5.

Patients with ABO incompatibility developed mild to moderate hemolysis, which was controlled with hydrocortisone, prednisolone, and antihistamines.

As a whole, the disease-free survival was the same for the groups who received BU (15 mg/kg), CY (200 mg/kg), ± ATG namely 72 versus 73%, but 27% of patients without ATG developed extensive chronic GVHD (Tables 2 and Table 3). The actuarial survival and disease-free survivals are shown in Figs 1and 2, respectively.

DISCUSSION

The first patient with thalassemia who was treated successfully with allogeneic BMT was performed in Seattle.[1] Among 30 patients with thalassemia who received BMT in the United State, the rate of event-free survival was 57%; recurrent thalassemia was observed in 24% of patients. These patients underwent transplantation over a period of 10 years in six centers using nine different preparative regimens.[2]

From 1981 to 1991, Giardini et al, transplanted 484 patients with beta-thalassemia major from HLA-identical donors: 88 of them died (18%), 41 patients rejected the graft (8%), and 355 were cured (73%).[3] They reported that the combination of BU, CY, and total body irradiation was toxic to thalassemic patients. They adopted a chemotherapy regimen in an attempt to eradicate thalassemia without the use of total body irradiation, because of the early toxicity and potential long-term effects in young children.[4]

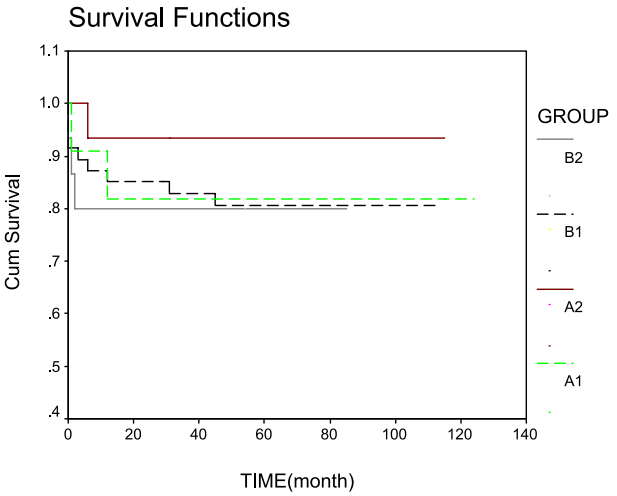

Fig 1. Kaplan-Meier projection for survival for 88 patients receiving transplants for the treatment of thalassemia with different regimens.

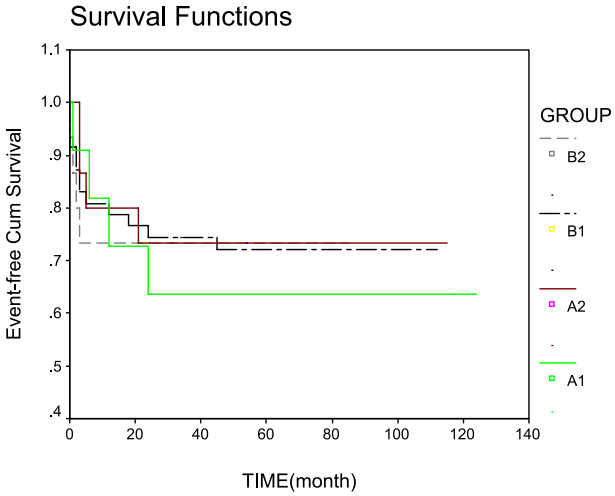

Fig 2. Kaplan-Meier projection for disease-free survival for 88 patients receiving transplants for treatment of thalassemia with different regimens.

Lucarelli et al used BU (16 mg/kg) and CY (200 mg/kg) on six thalassemic patients aged 6 month to 7 years (early stage of disease) with 50% of them dead due to transplant-related complications. By decreasing the dose of BU to 14 mg/kg, the actuarial survival was 86% (only one patient died) and the actuarial disease-free survival was 73% for 24 patients with five patients (20%) experiencing rejection.[4]

The preparative regimens used for transplantation in class I and II (BU, 14 mg/kg and CY, 200 mg/kg; protocol 6) were poorly tolerated by patients in class III as reported by Lucarelli et al.[5] The protocol of Thutschka et al. to reduce toxicity reduced the dose of CY to 120 mg/kg over 2 days without an apparent increase in graft rejection or malignancies,[6] but was associated with a relatively high rejection rate among thalassemic patients. In an attempt to reduce the probability of rejection AGT was administered during the peritransplant period (BU, 14 mg/kg, CY, 120 mg/kg, ALG, 100 mg/kg) to balance the decreased immunosuppressive effects of the BU-CY combination.[5]

Anti-lymphocyte serum was used for prevention of secondary disease (GVHD) as early as 1970 by Van Bekkum et al, and as an immunological conditioning agent in the same year by Floersheim and Ruszkienwicz, and even as the only conditioning agent for patients receiving marrow allografts by Mathe et al,[7] including one patient who suffered from thalassemia major.

A group in China used ATG 110 mg/kg over 11 days for all patients and increased the dose of BU from 14 to 16 mg/kg and CY 200 mg/kg for thalassemic patients younger than 10 years and CY 120 mg/kg for those older than 10 years to reduce the high rejection rate in southeast Asian patients. They reported an 85% disease-free survival with a follow-up of 13 to 42 months.[8]

ATG is a heteroantiserum (immunoglobulin G antibody) prepared by immunizing animals (horse, mouse) with pooled human thymocytes.[9] ATG is considered a potent immunosuppressive agent. There is a significant reduction in both the proportion and absolute number of peripheral blood lymphocytes expressing the activation antigen Tac (interleukin-2 receptor) and in the proportion of HLA-DR lymphocytes,[10] On the other hand, ATG is a differentiation-inducing agent; horse ATG induces growth and differentiation of normal bone marrow and terminal differentiation of the HL 60 cell line (AML-M3).[11] The bone marrow granulocyte colony-forming units increase after ATG treatment.[12] By adding ATG to the conditioning regimens, the incidence of severe acute and extensive chronic GVHD is less; it is regarded as a better conditioning therapy for HLA-mismatched transplants. As we reported previously,[12] and was seen among group A1 patients, the protocol BU (14 mg/kg plus CY 200 mg/kg), which was used by Lucarelli et al in class I and II patients, was associated with a relatively high rejection rate and partial chimerism in our class I to III patients. Since 1995, our protocol for BMT for most thalassemic patients includes BU (15 mg/kg), CY (200 mg/kg), and ATG (40 mg/kg), which has yielded good results.

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REFERENCES

1. Thomas ED, Buckner CD, Sanders JE, et al: Marrow transplantation for thalassemia. Lancet 2:227, 1982
2. Walters MC, Thomas ED. Bone marrow transplantation for thalassemia. The USA experience. Am J Pediatr Hematol Oncol 16:11, 1994
3. Giardini G, Angelucci E, Lucarelli G, et al: Bone marrow transplantation for thalassemia. Experience in Pesaro, Italy. Am J Pediatr Hematol Oncol 16:6, 1994
4. Lucarelli G, Galimberti M, Delfini C, et al: Marrow transplantation for thalassemia following busulphan and cyclophosphamide. Lancet 15:1355, 1985
5. Lucarelli G, Galimberti M, Polchi P, et al: Bone marrow transplant in adult thalassemia. Blood 80:1603, 1992
6. Thutschka PJ, Copela EA, Klein JP: Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. Blood 70:82, 1987
7. Mathe G, Amiel JL, Schwarzenberg L, et al: Bone marrow graft in man after conditioning, by antilymphocyte serum. Br Med J 2:131, 1970
8. Shing MMK, Yuen PMP, Li CK, et al: A more immunosuppressive pre-transplant conditioning may be required for Chinese patients with thalassemia. Bone Marrow Transplant 17:907, 1996
9. Hunter RF, Mold NG, Brain MR, et al: Differentiation of normal marrow and HL 60 cells induced by antithymocyte globulin. Proc Nati Acad Sci U S A 82:4823, 1985
10. Plantarias L, Gascon P, Bielory L, et al: Lymphocyte phenotype and lymphokines following anti-thymocyte globulin therapy in patients with aplastic anemia. Br J Hematol 66:437, 1987
11. Faillc A, Barrctt AJ, Balttrand N, et al: Effect of antilymphocyte globulin on granulocyte precursors in aplastic anemia. Br J Heamatol 42:341, 1979
12. Zakerinia M, Nourani Khojasteh H, Ramzi M, et al: Bone marrow transplantation for thalassemia in Shiraz. Transplant Proc 27:2659, 1995