BONE MARROW TRANSPLANTATION PROGRAM PEDIATRIC ONCOLOGY EXPERIENCE AT KING HUSSEIN MEDICAL CENTER

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ABSTRACT

Objective: To evaluate the pediatric bone marrow transplantation program at King Hussein Medical Center, between June 1997 to December 2002.

Methods: Fourteen children were transplanted with a median age of 9.5 years (range: 4-13 years), with the following diseases; Severe aplastic anemia (5), acute lymphoblastic leukemia (2), acute myeloid leukemia (4), chronic granulocytic leukemia (1), hyper-immunoglobulin M syndrome (1) and Fanconi's anemia (1).
All children and their donors were prepared before bone marrow transplantation according to our protocol.

Results: The patients engrafted at a median day +15 post transplant (range: 11-25 days). The mortality rate due to transplant-related toxicity was 14.3 % at +100 days and the overall disease-free survival was 78.6 % at a median follow-up period of 16.5 months post transplant (range: 1-66 months).

Conclusion: The results of the pediatric bone marrow transplantation program over the last few years were comparable to those reported from other centers in terms of mortality and morbidity. All these make it worthy to move on with the program.

Key words: Bone marrow transplantation, Hemorrhagic cystitis, Pediatric oncology.

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Introduction

Bone marrow transplantation (BMT) has been frequently used for treatment of different hematological and malignant diseases [1,2] and inherited metabolic disorders [3]. Hemopoietic stem cell transplantation (HSCT) involves the intravenous infusion of hemopoietic progenitor cells (bone marrow, peripheral blood stem cells, umbilical cord blood) to re-establish marrow function in a patient with damaged or defective bone marrow, and as a rescue after high dose chemotherapy for malignancy [4].

The first substantial series of BMT as a salvage procedure was reported by Thomas in 1977 who grafted 100 patients with relapsed leukemia from human leukocyte antigen (HLA)-matched siblings with depressing results [5]. Currently, more than 10,000 transplants are being performed annually worldwide. The success rate of the procedure has increased as a result of improving the supportive care, as well as the better understanding of the immunological changes accompanying the transplantation.

At King Hussein Medical Center (KHMC), we established a comprehensive protocol for allogeneic sibling bone marrow transplantation for both adults and children patients with hematological and malignant diseases. The co-ordination team included the senior members of the medical oncologists, hematologists, infectious diseases phycisians, microbiologists, immunologists, blood bank doctor, tissue typing specialists, pharmacist, and the nursing staff.

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The BMT program was started in 1996 utilizing a 2-bed unit. Transplants were performed on both adults and children. Between 1996 and 2002, a total of 72 pediatric and adult BMT were performed in this unit.

In February 2002, the BMT program was re-evaluated and indications for BMT were set as shown below:

1. Severe aplastic anemia (SAA)
2. Chronic granulocytic leukemia (CGL)
3. Acute myelogenous leukemia (AML), (poor risk group)
4. Acute lymphoblastic leukemia (ALL) in second complete remission (CR_2)
5. Immune deficiency disease; Severe combined immune deficiency (SCID), Hyperimmunoglobulin M (IgM) syndrome
6. β-thalassemia major (at a very young age)
7. Lymphoma in relapse

The objective of this report was to evaluate the experience and outcome of the pediatric allogeneic BMT at KHMC.

Methods

Fourteen pediatric patients underwent sibling allogeneic BMT at KHMC between June 1997 to December 2002. The age range was 4-13 years with a median of 9.5 years. There were 8 males and 6 females. HLA typing was done by the serological methods; the enzyme-linked immunosorbent assay (ELISA) for class I (HLA-A, B, C) and PCR for class II (HLA-DR, DQ). Eleven patients received bone marrow from fully HLA-matched donors and three patients from one antigen-mismatched donors. The following diseases were included for BMT: SAA (5), Fanconi anemia (1), ALL (2), AML (4), CGL (1), HyperIgM syndrome (1) as shown in Table I.

Patients with multiple leukemic relapses and patients with thalassemia major were excluded.

The conditioning regimen used was Busulfan (Bu) 16mg/kg body weight over 4 days and C yclophosphamide (Cyclo) 200 mg/kg over 4 days for AML, CGL, biphenotypic leukemia and hyper IgM immune deficiency syndrome. For relapsed ALL we used Bu: 16 mg/kg, Cyclo: 200 mg/kg and VP 16: 1000 mg/m^2 over 4 days, for aplastic anemia: anti-thymocyte globulin (ATG); 30 mg/kg daily for 3 days and Cyclo: 50 mg/kg daily for 4 days, and for Fanconi's anemia (FA), fludarabine 30 mg/m^2/day (six days). from day -10 to day -5, Cyclo: 10 mg/kg/day for 2 days from day -6 to day -5 and ATG: 30 mg/kg/day (3 days) from day -4 to day -2, methyl prednisolone 2 mg/kg/day from day -4 to day -2.

Prophylactic therapy against graft versus host disease (GVHD) was given to all patients in the form of Cyclosporin 3 mg/kg intravenously on day -1 and a short course of Methotrexate 15 mg/m^2 IV with folinic acid rescue on days +1, +3, +7 post BMT.

All patients received Acyclovir 500 mg/m^2 every 8 hours IV as an anti-cytomegalovirus (CMV) agent until de-isolation, and then continued on oral acyclovir until day +100 post BMT. They also received intravenous Gammaglobulin (IV Ig) at a dose of 500 mg/kg once weekly until de-isolation except for the patient with hyper IgM syndrome who continued on gammaglobulins every 3 weeks until day +180 post BMT.

Bone marrow was harvested under general anesthesia from all donors. The total nucleated cells (TNC) count was done during harvesting. The average TNC was 3.1 x 10^8 cells/kg of the patient. The range was 2.5-5.0 x 10^8 cells/kg.

Red blood cells were separated from the harvested bone marrow using the cell separator (Cobe Spectra) in patients with ABO incompatible blood. Bone marrow was infused via a central venous catheter without using a filter.

All patients were isolated in the unit from day 0 until engraftment which was assessed by an absolute neutrophil count of > 0.5 x 10^9/L for 3 consecutive days.

Monitoring

The oral and intravenous (IV) fluid intake plus the urine output were recorded daily by the nursing staff, and urinalysis was performed during and after cyclo administration. All patients and their families were asked to report any urinary symptoms, passage of red urine or clots. Accurate fluid balance during the administration of chemotherapy was closely watched and the medical staff was notified if no urine passed in eight hours.

Grading of hematuria: The diagnosis of hematuria was established on urinary sediment examination and the degree was assessed according to the number of red blood cells per high power field (RBC/HPF). The grading of hematuria was as follows:

Grade	RBC/HPF
0	0 - 10
1	10 - 100 (microscopic hematuria)
2	> 100 (gross hematuria)
3	Severe + clots

Results

Engraftment: All patients have engrafted at a median day + 15 post BMT (range: 11-25), including the two patients who died of severe hemorrhagic cystitis.

Mucositis: Grade 1-3 mucositis developed in 8 (57%) out of the 14 patients, which necessitated to give total parenteral nutrition (TPN) in the form of IV amino acids, vitamins and glucose.

Sepsis: Two patients; one with CGL and another with FA got *Staph epidermidis* septicemia post BMT and were treated successfully with antibiotics. The two patients who died from severe hemorrhagic cystitis got Gram-negative septicemia. Table II shows the complications and outcome of the children who underwent BMT.

Hemorrhagic cystitis: Five patients (35.7%) out of 14, developed late hemorrhagic cystitis (HC). Two patients;

no. 2 and 6, developed grade 2 HC and three patients; no. 8, 9 and 10, developed grade 3 severe HC. Patients with grade 2 were treated successfully with IV fluids hydration for few days. Patients with grade 3 required continuous fluid hydration, bladder irrigation, and cystoscopy to remove blood clots from the urinary bladder. Patient no. 8 was successfully managed and the HC resolved, but in the other two patients; (no. 9 and 10), the bleeding became worse and progressed to renal failure despite vigorous treatment with other measures such as antifibrinolytic agents, silver nitrate and bladder cautery. At the end, they developed Gram-negative septicemia and died due to multi-system failure.

Acute GVHD: Three patients (21.4 %) developed mild GVHD. Two of those; no. 3 and 12 had GVHD grade I (involving skin) and the third (no. 6) had GVHD grade II (skin + GI). All were treated with steroids and showed good response. None of them developed chronic GVHD. None of the 14 patients developed veno-occlusive disease (VOD) or CMV pneumonitis.

Mortality:

- Two patients died within 100 days post BMT (Patients no. 9 and 10) due to severe HC at 45 and 60 days post BMT, respectively. The mortality rate due to transplant-related toxicity was 14.3 % at 100 days post BMT.
- Patient no. 5 with severe aplastic anemia developed myelodysplastic syndrome (MDS) at 24 months post BMT, which progressed to AML and he died at 28 months post BMT due to progressed disease.
- The overall survival and disease-free survival was 78.6 % at a median follow-up of 16.5 months post BMT (range: 1-66 months).

Discussion

A comprehensive program for allogeneic sibling BMT was established at KHMC for both children and adults. All patients were selected satisfying the indications listed in Table I. The reason for that was because the unit was very small and the priority was given to patients with a better chance for survival. Patients with several relapses in ALL and AML during the course of their treatment were excluded, because the survival in such a group is not more than 20 % as stated in several reviews [6,7]. Also, the patient with CGL was transplanted in the chronic phase and not in the acute crisis or in leukemic transformation.

It was also decided not to transplant thalassemia children because we believed that they were highly sensitized from repeated blood transfusions and the majority were in class III (Pezero classification). The transplant in such a group of patients is very risky [8], so it was preferred to treat them by regular transfusions and desferroxamine (Desferal) administrations, as this regimen is carried out in many countries with good results. The Dutch experience showed that the probability of rejection was 55% among 11 patients who received a standard Bu/cyclo conditioning with or without ATG [9].

Four patients with AML were transplanted at the time of first remission. The reason why they were scheduled for transplantation was because they did not achieve remission after the first cycle of chemotherapy, so they were considered as a risky group. In patients achieving remission, BMT has been considered the treatment of choice if a matched sibling donor is available. Results of the analysis showed that allocation to BMT reduced the risk of relapse and it improved disease-free and overall survival [10].

One patient had immune deficiency; hyper IgM syndrome due to hereditary deficiency of the CD40-ligand, which is a rare congenital disease characterized by recurrent infections and a very low level of serum immunoglobulins (IgG, Ig A and IgE) and elevated IgM. He was on conservative treatment with antibiotics and regular IV Ig given once every three weeks. Experience of BMT in such cases is limited as a definitive treatment for this kind of syndromes. It was decided to transplant him from his HLA-matched sister, and he showed good clinical recovery where his serum IgG and IgM became within the normal limits for his age, while his IgA levels increased from undetectable values prior to BMT to go up to 43 and 73 mg/dl at 6 and 23 months post BMT, respectively. This is the first case in Jordan to be treated with BMT successfully.

The conditioning regimen used for malignant disease was Bu/Cyclo and not Cyclo/Total body irradiation (TBI). The reason for this was TBI was not available at the time of transplantation. The results of the French group demonstrated a superior outcome in patients treated with TBI/Cyclo compared to Bu/Cyclo in AML, but rather equivalent in CGL [11]. Our limited experience using Bu/Cyclo in AML is very good. Patients with SAA were conditioned with ATG and Cyclo, because they were very sensitized due to repeated blood transfusions. One patient with Fanconi's anemia was conditioned with non-myeloablative regimen using fludarabine, cyclo, and ATG. The allogeneic non-myeloablative BMT was much better tolerated in comparison with the side effect such as mucositis and hemorrhagic cystitis, following a standard myelo-ablative regimen (Bu/Cyclo). Now, allogeneic non-myeloablative stem cell and BM transplantation represents a new approach for safer treatment of malignant and genetically abnormal host hemopoietic cells [12].

High dose Acyclovir was used initially as a CMV prophylaxis regimen without hyperimmune anti-CMV immunoglobulins to prevent CMV infection. The reason behind that was the majority of patients were CMV-positive and it was difficult to get CMV-negative blood products. None of our patients developed CMV pneumonitis or related infections. Some investigators proved that hyperimmune immunoglobulins had no

beneficial effect as CMV prophylactic [13,14], but we did not study this in our patients.

The mortality rate due to transplant-related toxicity after 100 days post BMT was 14.3 %, and this is acceptable compared to international reported figures. Two patients were lost due to despite all measures that were taken to prevent hemorrhagic cystitis post Cyclo administration. The explanation for late HC is still unexplained and the hypothesis of viral etiology is one possibility [15-17]. Some authors in recent years reported CMV reactivation induced BK virus-associated late hemorrhagic cystitis after peripheral blood stem cell transplantation. In our center, we did not have the facility for complete viral studies, so the possible etiology of late hemorrhagic cystitis could not be explained [18].

We did not notice increased incidence of sepsis with bacteremia during the neutropenic period; this was due to the excellent nursing staff training in the unit. Fifty-seven percent of the patients developed mucositis grade 1-3, and for that they received analgesics in the form of oral morphine sulphate tablets or morphine IV. TPN was given due to loss of appetite or due to mucositis.

One child with severe aplastic anemia developed secondary MDS following successful BMT 24 months post transplant, then unfortunately his MDS transformed into AML after which the child died of progressive disease. It has been shown that BMT has a low but yet significant risk of secondary cancers. The literature on the incidence of secondary MDS post BMT is scanty. Armitage *et al* reported 4 out of 117 (3.4 %) incidence post stem cell transplantation for non-Hodgkin's lymphoma [16]. The secondary MDS have an unfavorable prognosis and survival is usually < 1 year and is probably related to the speed of evolving into acute leukemia [20].

In conclusion, the results of the pediatric BMT program at KHMC over the last few years are comparable to those reported from other centers in terms of mortality and morbidity. Our result of 78.6 % disease-free survival is promising so far, although it is too early to draw conclusions. BMT is a life saving procedure for many malignant and non-malignant hematologic diseases despite the complications. All these make it worthy to move on with the program.

Table 1. Characteristics of patients and conditioning regimens used.

Patient	Sex	Age (years)	Disease	HLA-match	Conditioning regimen
1	F	8	AA	Compatible	Cyclo, ATG
2	M	9	AML	1 antigen mismatch	Bu/Cyclo
3	M	13	CGL	1 antigen mismatch	Bu/Cyclo
4	F	4	AML	Compatible	Bu/Cyclo
5	M	7	AA	1 antigen mismatch	Cyclo, ATG
6	M	10	Hyper IgM Syndrome	Compatible	Bu/Cyclo
7	F	13	AML	Compatible	Bu/Cyclo
8	M	11	AL Biphenotypic	Compatible	Bu/Cyclo
9	F	13	ALL-CR_2	Compatible	Bu/Cyclo + VP 16
10	F	13	AA	Compatible	Cyclo, ATG
11	M	4	AML	Compatible	Bu/Cyclo
12	M	7	AA	Compatible	Cyclo, ATG
13	M	13	AA	Compatible	Cyclo, ATG
14	F	7	FA	Compatible	Flud. Cyclo, ATG

AA: Aplastic anemia, AML: Acute myeloid leukemia, CGL: Chronic granulocytic leukemia, ALL-CR_2: Acute lymphoblastic leukemia in second complete remission, FA: Fanconi's anemia, BU: Busulfan, Cyclo: Cyclophosphamide, ATG: Anti-thymocyte globulin, Flud: Fludarabine.

Table II. Complications and outcome of children who underwent BMT.

Patient No.	Mucositis	Sepsis	GVHD	H C	Outcome	Follow-up Period post BMT (in months)
1	-	-	-	-	Alive	66
2	-	-	-	+	Alive	40

3	+	+	+	-	Alive	39
4	-	-	-	-	Alive	29
5	-	-	-	-	Died	28
6	-	-	+	+	Alive	25
7	+	-	-	-	Alive	18
8	+	-	-	+	Alive	15
9	+	+	-	+	Died	1.5
10	+	+	-	+	Died	2
11	-	-	-	-	Alive	8
12	+	-	+	-	Alive	7
13	+	-	-	-	Alive	2.5
14	+	+	-	-	Alive	1

Note: Patients with follow up until Dec 2002. Median follow-up: 16.5 months, range: (1-66)

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