Allogeneic Bone Marrow Transplantation in an Osteopetrosis Patient: First Report in Thailand

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The first successful allogeneic bone marrow transplantation in Thailand was carried out in an aplastic anemia patient by Issaragrisil et al. in 1987. Up until now over 200 patients in Thailand have undergone either allogeneic or autologous stem cell transplantation. Most Thai patients who underwent stem cell transplantation had malignancies, hemoglobinopathies, and aplastic anemia. Osteopetrosis is an autosomal recessive disorder that results from failure of the osteoclast to resorb bone and cartilage. Due to accumulation of excess bone, patients develop bone marrow failure and entrapment of nerves. Most patients fail to grow and die at an early age from anemic bleeding and infection. Splenectomy, steroids, parathyroid hormones, and cytokine therapy have been tried for this disorder, however, response has been minimal and transient. The only curative therapy for osteopetrosis is an allogeneic bone marrow transplantation. To the best of our knowledge, this report is the first of a successful allogeneic bone marrow transplantation in an osteopetrosis patient in Thailand.

PATIENT AND METHOD

The diagnosis of osteopetrosis was made in August 1995 in 5-year-old male presenting with visual impairment. Physical examination revealed hepatosplenomegaly. Laboratory findings demonstrated low hemoglobin level and leukoerythroblastic anemia. Bone x-ray findings demonstrated uniformly dense, homogeneous and sclerotic bone with an absence of corticomedullary junctions as Fig. 1a. Bone marrow biopsy (Fig. 2) showed diffusely dense bone with loss trabecular and lamellar pattern, persistent of cartilage. Osteoclasts were focally increased. The bone marrow spaces were markedly re-

SUMMARY We described the successful allogeneic matched sibling bone marrow transplantation (BMT) in a 5-year-old Thai boy in whom osteopetrosis was diagnosed on the basis of anemia, thrombocytopenia, leukoerythroblasticosis, sclerotic bone, hepatosplenomegaly, and visual deficit from an encroachment of cranial nerve foramina. The preparative regimen included 4 days of busulfan 4mg/kg/day, and 4 days of cyclophosphamide 50 mg/kg/day. Complete hematopoietic engraftment and no evidence of graft versus host disease were shown after BMT. Complete hematologic findings were corrected. His hematopoietic chimerism was changed to that of his donor. Post BMT, he has no hepatosplenomegaly. His bone radiographic findings revealed normal after BMT. Bone marrow biopsy showed normalized bone and bone marrow matrix. However, his vision remained impaired. We believe that this is the first case of successful bone marrow transplantation in an osteopetrosis patient in Thailand.
Fig. 1 Bone x-ray. a: left femur, showing uniformly dense, homogeneous and sclerotic with absence of corticomedullary junction, b: post transplant bone x-ray left femur (day +386) showing improvement with medullary canal and distinct corticomedullary border.

Fig. 2 Bone marrow biopsy. a: (HE x 20) showing diffusely dense bone with loss of trabecular pattern and reduction of the intervening marrow spaces, b: (HE x 50) showing persistence of cartilage, increased osteoclasts with no evidence of osteoclast resorption of bone.
duced. These were characteristic of osteopetrosis. He underwent allo- 
geneic bone marrow transplant in December 1997. The conditioning 
regimen consisted of busulfan 4 mg/kg/day and cyclophosphamide 
50 mg/kg/day for 4 days which continued after busulfan adminis- 
tration. Bone marrow stem cells 
were harvested from his brother 
sibling and infused to the patient 
after a conditioning regimen. Both 
class I and class II HLA of his 
brother were identical to his HLA. 
The number of mononuclear cells 
which were infused to the patient 
were 5.1 x 10^8 cells/kg and the 
number of CD34 cells which were 
infused to the patient were 6.27 x 
10^6 cells/kg. Graft versus host pro- 
phylaxis was with cyclosporin A at 
a dose 3 mg/kg IV q 12 h until day 
+30 and orally for 100 days and 
methotrexate (15 mg/m^2 IV on day 
+1, and 10 mg/m^2 on days +3, +6, 
and +11).

To perform a DNA finger- 
print to detect chimerism pre- and 
post BMT in the patient, 9 short 
tandem repeat (STR) loci: 
D3S1358, vWA, FGA, THO 1, 
TPO X, CSF 1 PO, D5S 818, 
D13S317, D7S820, and the seg- 
ment of X-Y homologous gene 
Amelogenin were co-amplified in a 
single tube. This amplification kit 
was purchased from PE Applied 
Biosystem (Perkin Elmer, USA). In 
the kit, one primer of each locus- 
specific primer was labeled with 
either the 5-FAM, JOE or NED 
NHS-ester dye. Amplification was 
carried out in thin-walled Micro- 
Amp tubes (Perkin Elmer) in a 
GeneAmp PCR system 2400 
(Perkin Elmer), using the following 
conditions: pre-heating at 95°C for 
13 minutes then 94°C for 1 minute, 
59°C for 1 minute and 72°C for 1 
minute for 28 cycles and followed 
by 60°C for 45 minutes. The am- 
plified products were separated by 
automated capillary electrophoresis 
(Applied Bio-system automated 
DNA sequencer model 310). DNA 
profiles were generated using 
Genescan and Genotype software.

RESULTS

The patient’s course of 
transplant procedure was unevent- 
ful. He achieved engraftment with 
an absolute neutrophil count of 
> 0.5 x 10^9/l at day +15 and time to 
unsupported platelet count of 25 x 
10^9/l at day +70. He did not devel- 
op acute or chronic graft versus 
host disease. On day +127, bone 
marrow biopsy was performed and 
showed evidence of virtual nor-
malization of the bone, the bone marrow matrix markedly reduced and improvement in the marrow space with normal hematopoietic precursors (Fig. 3). Clinically, there was remarkable reduction in the size of liver and spleen. However, his vision remained impaired. Radiologically, day +386 post BMT the bone density post-transplant showed improvement with apparent of medullary canals and distinct corticomedullary borders (Fig. 1b). A DNA fingerprint was performed to detect chimerism. There was evidence of chimerism of hematopoietic cells having been completely changed from recipient cells to donor cells (Figs. 4-6). For example, at short tandem repeat (STR) locus FGA in Fig. 4, the genotype profile of the donor was 19 and 21 whereas the recipient’s pre-BMT was 19 and 22. On December 25, 1997, the recipient’s genotype gradually decreased on December 31, 1997 and finally on January 9, 1998, the recipient’s genotype was identical to the donor’s.

**DISCUSSION**

Osteopetrosis is a rare inherited disorder characterized by generalized skeletal sclerosis that occurs in various mammals including humans. Osteopetrosis is a result of dysfunction of osteoclasts, the multinucleated giant cells that resorb bone and mineralized cartilage. The osteoclast is a specialized macrophage derived from the bone marrow stem cells. The inability to resorb and remodel bone due to osteoclast dysfunction, in the presence of normal bone formation by osteoclasts, results in the deposition of excessive mineralized osteoid and cartilage. All bones are uniformly dense, sclerotic and radiopaque. Medullary cavities are absent from a long bone radiograph. Bone biopsy reveals encroachment of medullary cavities by bone and mineralized cartilage, thick trabeculae, and decreased medullary spaces. Encroachment of marrow spaces leads to extra-medullary hematopoesis, progressive hepatosplenomegaly, and hypersplenism. The result is anemia with reticulocytosis, leukoerythroblastosis, and thrombocytopenia. Encroachment of cranial foramina leads to retinal atrophy, which progresses to blindness, auditory nerve damage, and oculomotor and facial nerve palsies. Several therapeutic strategies have been used in the treatment of osteopetrosis. Limited and transient improvement has been reported with such therapeutics as steroids and/or splenectomy to increase red cell and platelet survival, low calcium and high phosphate diet to reduce sclerosis, and high dose calcitriol and parathyroid hormone to enhance osteo-

![Fig. 4 DNA profiles of recipient and donor at various intervals evaluated for short tandem repeat (STR) alleles of loci D3S1358, vWA and FGA, respectively. A and B refer to pre-BMT allelic profiles of recipient and donor, respectively. C, D, and E refer to allelic profiles of recipients (post BMT) on December 25 and 31, 1997 and January 9, 1998, respectively.](image-url)
Bone marrow transplantation is curative in patients with osteopetrosis, which implies that osteoclast cells are transplantable. Ballet et al.\textsuperscript{14} reported the first BMT for osteopetrosis. A 3-month-old infant girl was transplanted without immunosuppression with marrow from a HLA identical 2-year-old sister. Although durable engraftment was not demonstrated, radiological and other evidence for significant bone resorption was present. Coccia et al.\textsuperscript{7} reported a 5-month old girl transplanted from her HLA-identical, mixed lymphocyte culture-compatible brother after preparation with cyclophosphamide (CY) (200 mg/kg) and modified total body irradiation (TBI) (400 cGy with head and lung shielding). Engraftment was documented by chromosome analysis. Anemia, thrombocytopenia, leukoerythroblastosis, and metabolic abnormalities were corrected within 12 weeks of BMT. Serial radio-
graphs revealed bony remodeling and new nonsclerotic bone formation. However, subsequent follow-up showed progressive loss of the graft. Several studies subsequently reported BMT in osteopetrosis patients. Most of the reported patients have been prepared with busulfan (BU) either 2 mg/kg/day for 4 days or 4 mg/mg/day for 4 days followed by CY at 50 mg/kg/day for 4 days. Engraftment was prompt, with development of hematopoietic mixed chimerism in most children. Failure of engraftment has been reported only with mismatched, T cell depleted grafts. Late graft failure has been reported in at least 3 well-studied patients.

Here we reported a successful bone marrow transplantation in an osteopetrosis patient. To the best of our knowledge, this is the first report of a successful BMT in such a patient in Thailand. We gave a preparative regimen to this patient with BU at a dose of 4 mg/kg/day for 4 days followed by CY 50 mg/kg/day for 4 days as previously described. All hematologic and radiographic abnormalities, and hepatosplenomegaly could be corrected. However, his vision remained impaired. A number of patients with osteopetrosis who have undergone BMT have been reported to have hypercalcemia complicating their post-BMT course. Our patient did not experience this complication. At present, 1 year after BMT, he has no hepatosplenomegaly, and blood counts and chemistries are normal. His bone radiographic is normal. He is intelligent.

Allogeneic BMT is considered the only curative modality of therapy available so far for patients with osteopetrosis. BU/CY is an effective preparative regimen in this group of patients with osteopetrosis. If BMT is undertaken at an early age, the neurosensory deficits that are expected to develop with time in this disease can be prevented, and in certain cases improvement can occur if there is a partial impairment of vision or hearing abilities. Cord blood stem cell transplantation should be considered for this group of patients when an HLA-identical sibling is not available.

REFERENCES