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

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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, leukoerythroblastosis, 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.**

The first successful allogeneic bone marrow transplantation in Thailand was carried out in an aplastic anemia patient by Issaragrisil *et al.*[1] 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 transplantion had malignancies, hemoglobinopathies, and aplastic anemia.[2,3] Osteopetrosis is an autosomal recessive disorder that results from failure of the osteoclast to resorb bone and cartilage.[4] 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.[5,6] The only curative therapy for osteopetrosis is an allogeneic bone marrow transplantation.[7,8] 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-

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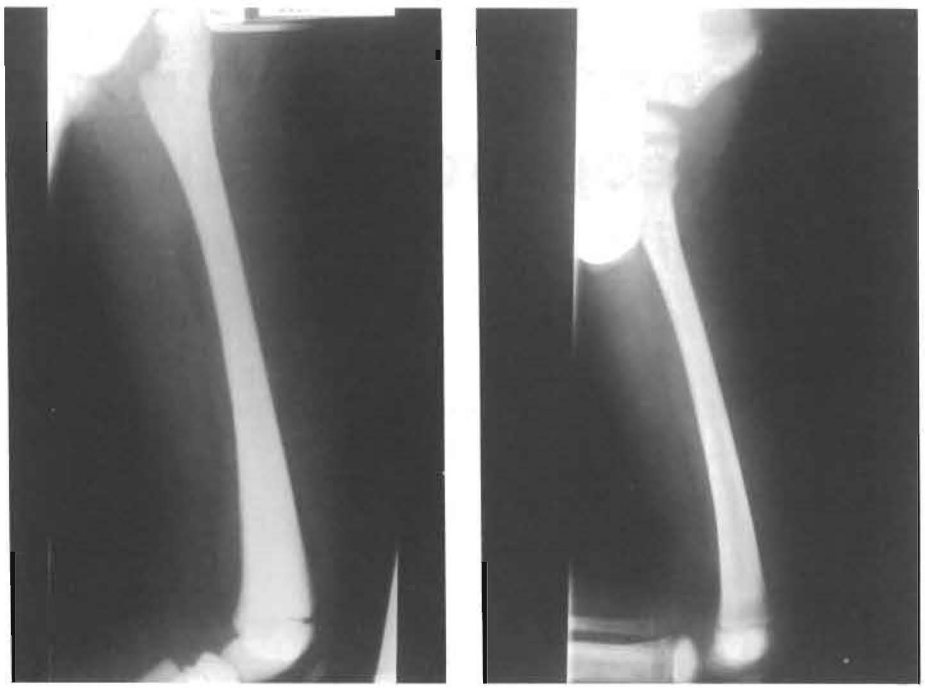

Fig. 1 Bone x-ray. a: left femur, showing uniformly dense, homogeneous and sclerotic with absence of corticomedullary junction, b: post transplant bone x-ray left femur (day +386) showing improvement with medullary canal and distinct corticomedullary border.

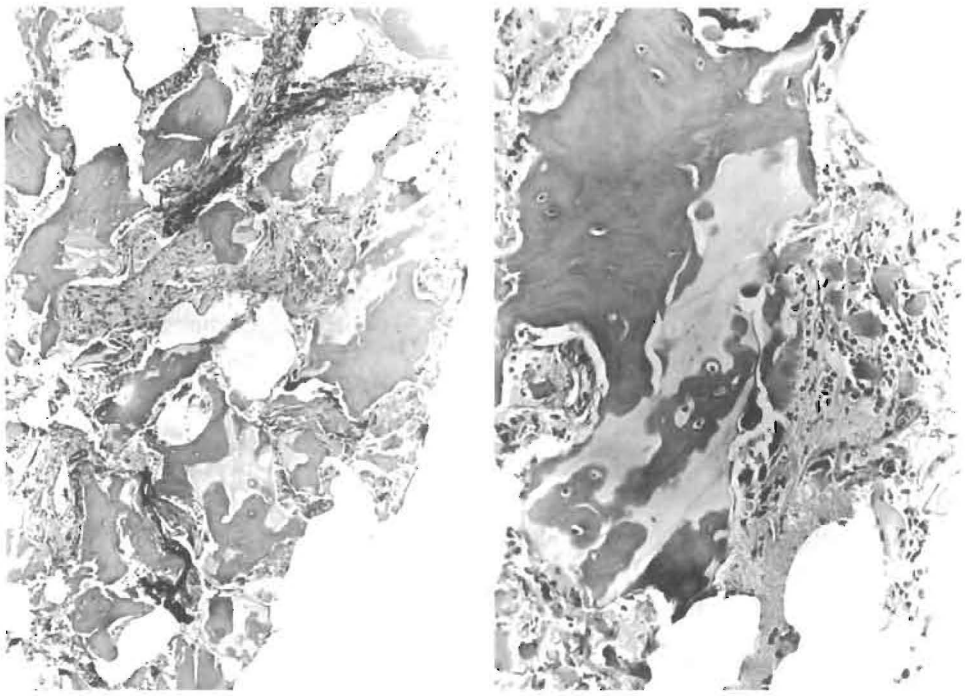

Fig. 2 Bone marrow biopsy. a: (HE x 20) showing diffusely dense bone with loss of trabecular pattern and reduction of the intervening marrow spaces, b: (HE x 50) showing persistence of cartilage, increased osteoclasts with no evidence of osteoclast resorption of bone.

duced. These were characteristic of osteopetrosis. He underwent allogeneic 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 administration. 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 prophylaxis 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 fingerprint 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 segment 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 amplified 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 uneventful. 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 develop acute or chronic graft versus host disease. On day +127, bone marrow biopsy was performed and showed evidence of virtual nor-

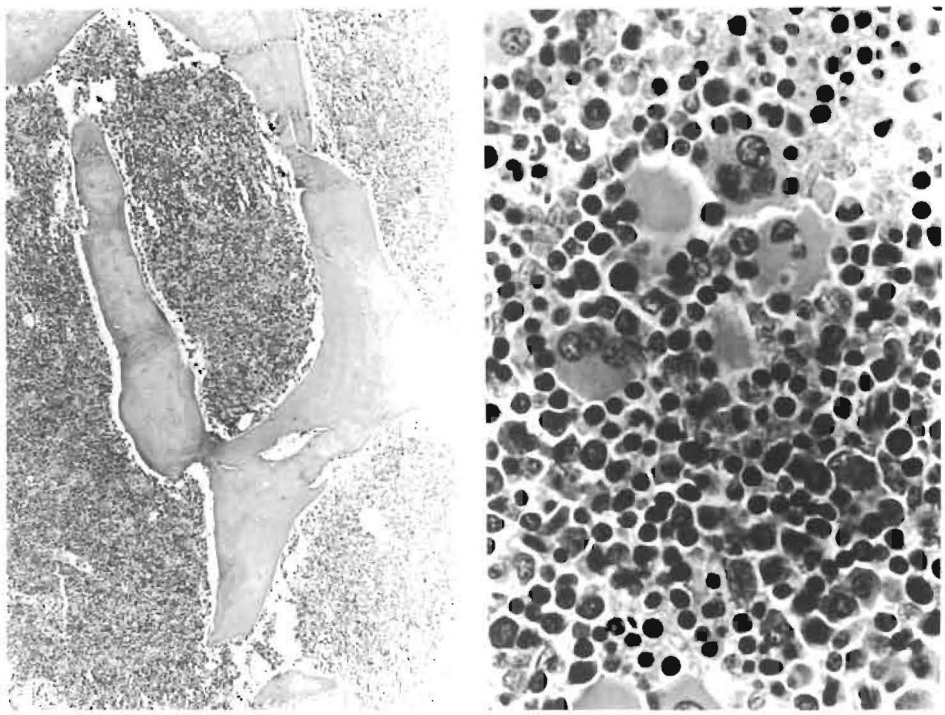

Fig. 3 Post transplant bone marrow biopsy (day +127). a: (HE x 20) and b: (HE x 200) showing normal bone trabecular pattern, hypercellular marrow with active hematopoiesis.

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.

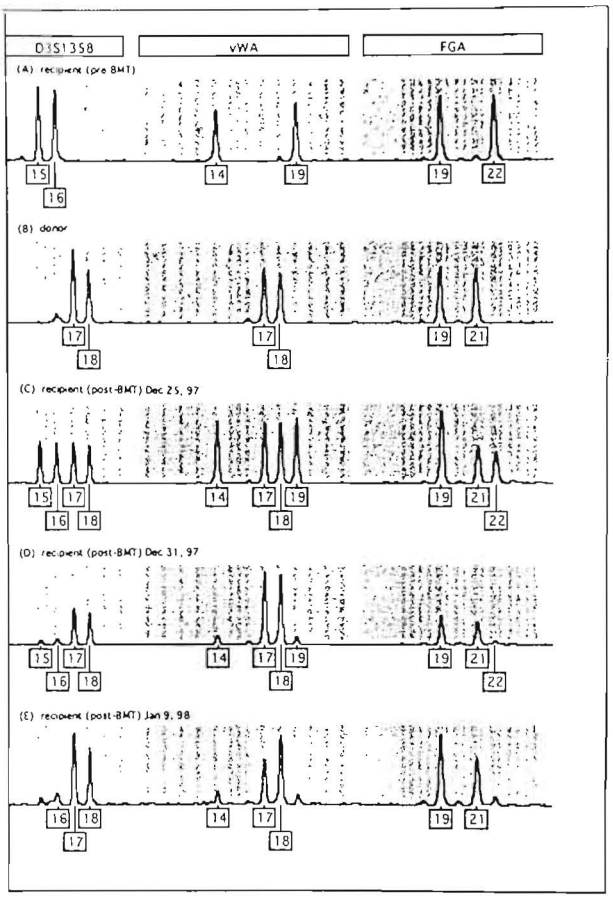

Fig. 4 DNA profiles of recipient and donor at various intervals evaluated for short tandem repeat (STR) alleles of loci D3S1358, vWA and FGA, respectively. A and B refer to pre-BMT allelic profiles of recipient and donor, respectively. C, D, and E refer to allelic profiles of recipients (post BMT) on December 25 and 31, 1997 and January 9, 1998, respectively.

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.[4] 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.[9] Encroachment of marrow spaces leads to extramedullary hematopoiesis, 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.[10] 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-

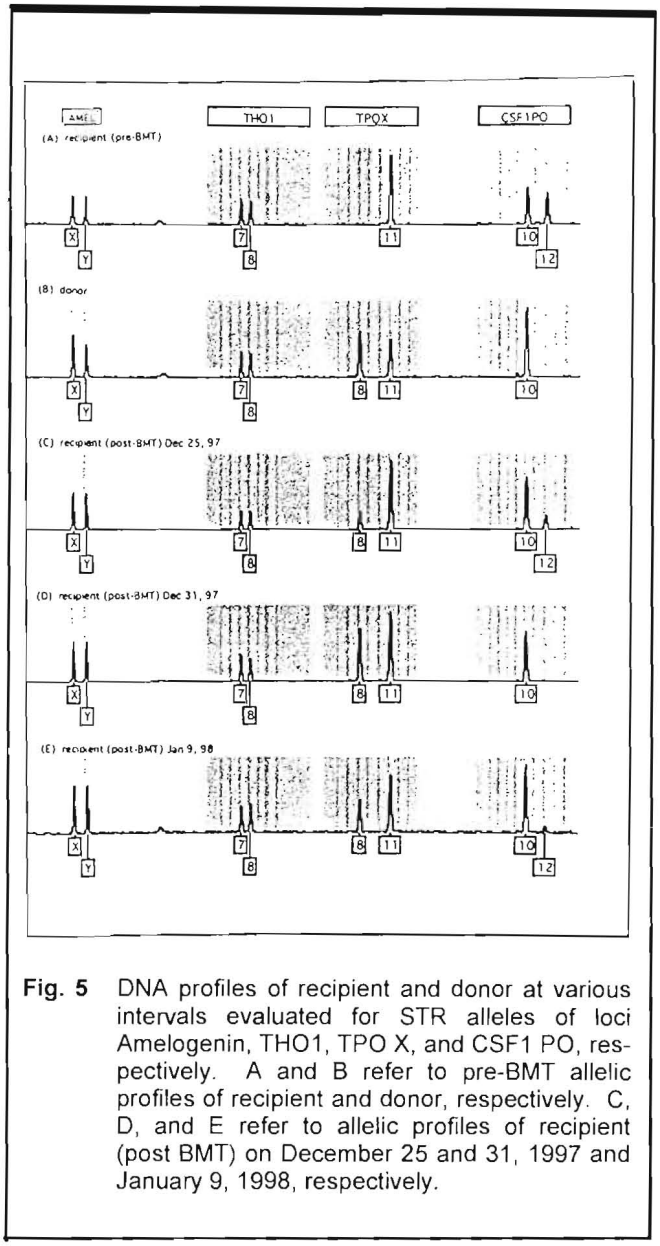

Fig. 5 DNA profiles of recipient and donor at various intervals evaluated for STR alleles of loci Amelogenin, THO1, TPO X, and CSF1 PO, respectively. A and B refer to pre-BMT allelic profiles of recipient and donor, respectively. C, D, and E refer to allelic profiles of recipient (post BMT) on December 25 and 31, 1997 and January 9, 1998, respectively.

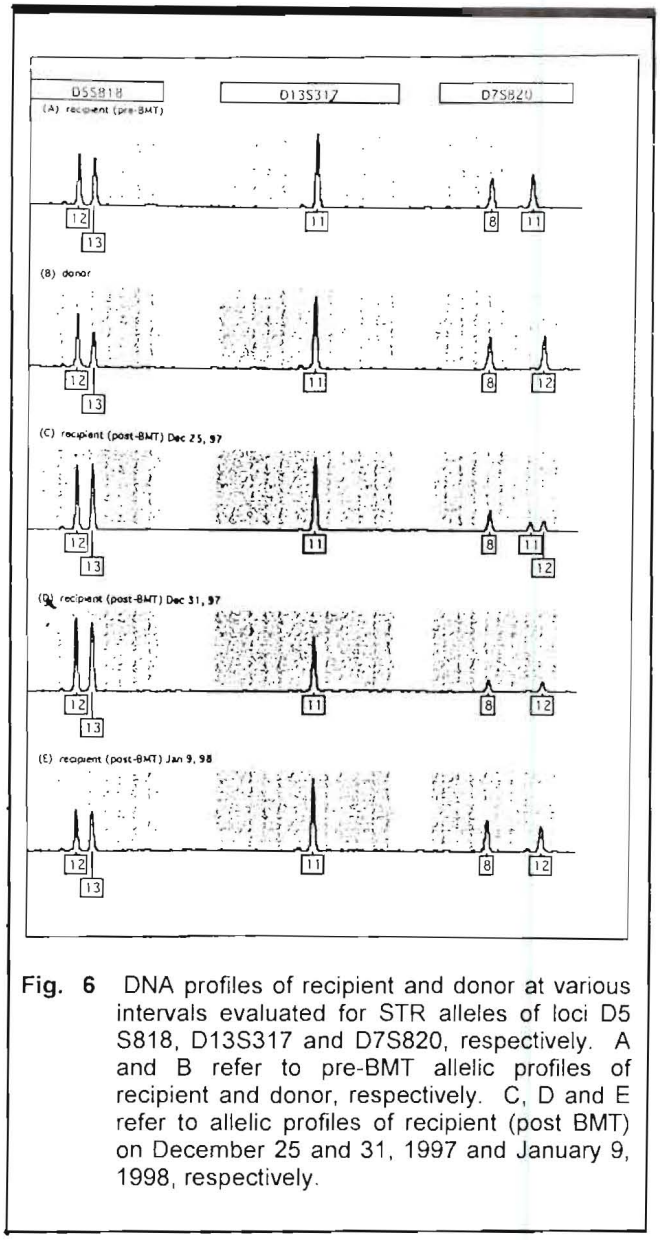

Fig. 6 DNA profiles of recipient and donor at various intervals evaluated for STR alleles of loci D5 S818, D13S317 and D7S820, respectively. A and B refer to pre-BMT allelic profiles of recipient and donor, respectively. C, D and E refer to allelic profiles of recipient (post BMT) on December 25 and 31, 1997 and January 9, 1998, respectively.

clastic function.[5,6] Some reports described the cure of osteopetrosis in both op/op mouse and the tl/tl rat with infusions of recombinant human macrophage stimulating factor (M-CSF).[11,12] However, the role of M-CSF in the therapy of humans with osteopetrosis is still unclear. Walker[13] demonstrated that hematopoietic engraftment of bone marrow and new cells can restore bone resorption in osteopetrotic mice. Bone marrow transplantation is curative in patients with osteopetrosis, which implies that osteoclast cells are transplantable. Ballet *et al.*[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.*[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.[7] 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.[8,15] 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.[7,16,17]

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.[18] 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.[19]

REFERENCES

1. Issaragrisil S, Chandanayingyong D, Suvatte V, *et al.* Bone marrow transplant for severe aplastic anemia: the first case report in Thailand. J Med Assoc Thailand 1987; 70: 160-7.
2. Issaragrisil S. Bone marrow transplantation in Thailand. Bone Marrow Transplant 1994; 13: 721-3.
3. Jootar S, Chancharunee S., Ungakanont A., Tanapothiwirat W., Chiewsilp P. Bone marrow transplantation in Ramathibodi Hospital: progress report Asian Pac J Allergy Immunol 1992; 10: 117-22.
4. Key LL. Osteopetrosis, a genetic window into osteoclast function. CTC series: cases in metabolic bone disease. New York: Triclinica Communications 1987; 1-12.
5. Reeves JD, Huffer WE, August CS, Hathaway WE, Kerper M, Walters CE. The hemopoietic effects of prednisone therapy in four infants with osteopetrosis. J Pediatr 1979; 94: 210-4.
6. Glorieux FH, Pettifor JM, Marrie PJ, *et al.* Induction of bone resorption by parathyroid hormone in congenital malignant osteopetrosis. Metab Bone Dis 1981; 3: 143-50.
7. Coccia P, Krevit IO, Cervenka J, *et al.* Successful bone marrow transplantation for infantile malignant osteopetrosis. N Engl J Med 1980; 13: 701-8.
8. Solh H, Da Cunha AT, Giri N, *et al.* Bone marrow transplantation for infantile malignant osteopetrosis. J Pediatr Hematol Oncol 1995; 17: 350-5.
9. Kolawole TM, Hawass ND, Patel PJ, *et al.* Osteopetrosis; some unusual radiographic features with a short review. Eur Radiol 1988; 8: 89-95.
10. Teitebaum SL, Coccia PF, Brown DM, Kahn AJ. Malignant osteopetrosis: a disease of abnormal osteoclast proliferation. Metab Bone Dis Rel Res 1981; 3: 99-105.
11. Felix R, Cecchini MG, Fleisch H. Macrophage colony stimulating factor restores in vitro bone resorption in the *op/op* osteopetrosis mouse. Endocrinology 1990; 127: 2592-4.
12. Marcks SC, Wojtowicz A, Szperl M, *et al.* Administration of colony stimulating factor-1 corrects some macrophage, dental, and skeletal defects in an osteopetrosis mutation (toothless, tl) in the rat. Bone 1992; 13: 89-93.
13. Walker DG. Bone resorption restored in osteopetrosis mice by transplant of normal bone marrow and spleen cells. Science 1975; 190: 784-5.
14. Ballet JJ, Griscelli C, Coutis C, Milhaud G, Maroteaux P. Bone marrow transplantation in osteopetrosis. Lancet 1977; 2: 1137.
15. Fischer A, Friedrich W, Levinsky, Vossen J, Griscelli C, Kubanek B. Bone-marrow transplantation for immunodeficiencies, osteopetrosis, and Fanconi's anemia: A European Survey. Lancet 1986; 1: 1080-4.
16. Sorell M, Kappor N, Kirkpatrick D, *et al.* Marrow transplantation for juvenile osteopetrosis. Am J Med 1981; 70: 1280-7.
17. Schroeder RE, Johnson FL, Silberstein MJ, *et al.* Longitudinal follow up of malignant osteopetrosis by skeletal radiographs and restriction fragment length polymorphism analysis after bone marrow transplantation. Pediatrics 1992; 90: 986-9.
18. O'Reilly RJ, Brochstein J, Dinsmore R, Kirkpatrick D. Marrow transplantation for congenital disorders. Semin Hematol 1984; 21: 188-221.
19. Gluckman E, Rocha V, Chastang C. The Eurocord group. European results of unrelated cord blood transplant. Bone Marrow Transplant 1998; 21. (Suppl. 3): 87-91.