RESEARCH PAPER

Recombinant Macrophage Targeted Enzyme Replacement Therapy for Gaucher Disease in India

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Objective: Gaucher disease in India has been reported only in a few case reports from India. The aim of the study was to assess the response to enzyme replacement therapy in Indian patients with Gaucher disease.

Design: Retrospective analysis of patients receiving CHO-derived recombinant macrophage-targetted glucocorebrosidase.

Setting: Five centers from India with experience in treating lysosomal storage disorders.

Patients: The diagnosis of Gaucher disease was confirmed by low glucocerebrosidase levels, though it was first made on splenectomy in 8 and on bone marrow examination in 9 patients. Twenty five of 52 patients diagnosed with Gaucher disease (17 Type I, 8 mild Type III) received treatment for >6 months. Indications for treatment included symptomatic anemia, thrombocytopenia, organomegaly, bone disease or mild neurological symptoms leading to impairment of quality of life. Patients with significant neurological involvement were excluded. The drug infusions were given intravenously every 15 days.

Main Outcome measures: Hemoglobin, platelet counts, liver and spleen volumes and growth parameters.

Results: 22 of the 25 children who survived were analyzed. After 6 months of treatment, the mean (range) increase in hemoglobin was 1.5 (-3.4 to 6.1) g/dL (*P*=0.01) and in platelet count was 32 x 10^9/L (-98.5 x 109 to 145.5 x10^9) /L (*P*=0.02). The mean (range) increase in weight was 3 kg (-5.6 to 10.5) (*P*=0.04) and in height was 7.1 cm (0 to 26.5) (*P*=0.0003). Liver size decreased by a mean (range) of 38.5% (- 5.5 to 86.7) (*P*=0.0003) and the spleen size by 34.8% (0 to 91.7) (*P*=0.004). All patients had improvement in bone pains and in 2 patients, neurological symptoms improved with others remaining static.

Conclusions: This is the first reported cohort of patients in India reporting our experience with imiglucerase enzyme replacement therapy for treatment of Gaucher Disease in India.

Key words: *Children, Gaucher disease, Imiglucerase, India, Lysosomal storage disorder, Treatment.*

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Gaucher disease (GD) is the most prevalent lysosomal storage disorder and has served as a prototype for genetic/ phenotypic delineation as well as therapeutic innovation. The metabolic defect is an inherited deficiency of lysosomal acid β-glucosidase due to mutations in the GBA1 gene [1]. The result is an accumulation of glucocerebroside in the lysosomes of macrophages and a complex multisystemic phenotype involving the liver, spleen, bone marrow, skeleton and the lungs. Gaucher disease is classified into three broad phenotypic

categories depending on the absence or presence of neurological involvement; although overall it represents a continuum of disease spectrum [2-5].

Gaucher disease is pan-ethnic but type 1 GD is most prevalent in Ashkenazi Jews [2]. An increased incidence of neuronopathic Gaucher disease has been reported from other countries [6,7] but the frequency and phenotypic spectrum of Gaucher disease in India is currently unknown. There have been isolated case reports of GD in India [8-11]. Recently, we have been able to treat patients in India with Gaucher disease with recombinant macrophage-targeted enzyme replacement therapy (ERT) through the Humanitarian Program of Genzyme Corporation. We herein report our experience of 25 patients with GD in India. This is also the first description of the phenotypic spectrum of GD in India.

METHODS

Fifty-two patients were diagnosed with GD over a period of 10 years at the recruiting centers across India. Examination of patients included a general systemic examination with neurological evaluation including an ophthalmologic examination. Forty-three of the 52 patients were evaluated for enzyme replacement therapy (ERT). Nine patients had mild, stable disease that did not require immediate ERT. The indications for enzyme replacement therapy were symptomatic anemia (i.e., requiring blood transfusion), symptomatic thrombocytopenia (mainly manifesting as epistaxis and easy bruisability), organomegaly or symptomatic bone disease and/or failure to thrive and/or significant impairement of quality of life. Patients with significant neurological involvement were excluded.

The enzyme replacement therapy consisted of CHO-derived recombinant macrophage-targeted glococerebrosidase (Imiglucerase, Cerezyme, Genzyme Coroporation). Freeze-dried recombinant enzyme was diluted in 0.9% NaC1 to a total volume of 100 mL and infused through a blood transfusion filter over 1 to 2 hours. An initial test dose of 5 mL was given. Initially, patients received enzyme dose of 60U/kg every 15 days. Subsequently, the dose was adjusted based on response to therapy.

The candidacy of patients to receive ERT was evaluated by the IMAB (Indian Medical Advisory Board) that included six expert physicians from centers across India, international experts on the disease, and representatives of Genzyme corporation. Enzyme therapy was initiated after informed consent was obtained. Before initiating enzyme therapy, patients had physical examination, laboratory studies including complete blood count, and a liver profile. Radiological studies included ultrasonography or CT scan of the abdomen to determine liver and spleen volumes, and long bone surveys to study bone deformities and densities. Patients who were enrolled in the previous one-year, also underwent chest *X*-ray and 2D echo to rule out lung parenchymal involvement and pulmonary hypertension, respectively. Patients were monitored every 6 months for their height, weight, hemoglobin, platelet count, liver and spleen sizes, and subjective assessment of bone pains, neurological symptoms and quality of life.

The hemoglobin, platelet, height and weight changes were analyzed using the paired student t test. The spleen and liver volumes were calculated in terms of percentage changes and their means were analyzed using the paired student *t* test.

RESULTS

Of the 22 patients currently receiving ERT for > 6 months, 16 exhibited Type I and six had Type III Gaucher disease. There were 15 males and 7 females, median age was 3.6 years (range, 1.6 to 26 years). The diagnosis of GD was first suspected on pathology of splenectomy specimens in 8 patients, in 9 patients on bone marrow examination showing typical Gaucher cells and in the remaining 5 patients, by direct determination of acid glucosidase activity in peripheral blood leucocytes. All patients underwent confirmation of diagnosis by demonstration of low glucosidase activity in peripheral blood leucocytes. The mean enzyme level was 10% (0-75%) of the lower limit of the normal range. There was history of consanguinity in parents of 11 patients (50%).

Of the 43 patients deemed suitable for ERT, 25 patients received treatment for ≥6 months. Three of

these 25 patients died within mean period of 22 months (range 15 to 36 months) of start of ERT in spite of receiving therapy and have been described separately.

Response to treatment was analyzed retrospectively from the data available of patients who were treated for more than or equal to 6 months. All patients had severe splenomegaly and/or hepatomegaly, as well as variable degrees of anemia and thrombocytopenia. Six patients (27%) had mild neurological involvement consistent with Type III Gaucher disease.

Hematological responses: Anemia was noted in all patients at the initiation of treatment (100%). The mean (range) baseline hemoglobin was 8.4 (3.8 to 11.2) g/dL. 11 patients had received blood transfusions prior to the start of treatment.

Thrombocytopenia was noted in 18 out of 22 patients at the initiation of treatment (82%) and the mean (range) baseline platelets were 116×109 (50-400×10^9)/L. The platelet levels of 11 patients completely normalized during the course of treatment. In 8 patients treated for more than a year, for an average of 2.5 years (1.5-10 years), the mean (range) increase in hemoglobin was 3.75 g/dL (*P*=0.005) and the mean (range) increase in platelets was 183×10^9/L (*P*=0.0007). The mean change in hemoglobin and platelets is shown in the ***Table* I**.

Weight and height changes: Two patients reported a loss of weight. The changes in weight and height are shown in the ***Table* II**.

In 8 patients who were treated for more than a year, for a mean of 2.5 years (1.5-10 years), the mean (range) increase in weight was 12.9 kg (-4.4 to 23.5) (P=0.006), and the mean (range) increase in height was 34.1 (1-67) cm (*P*=0.0003).

Liver size decreased over a mean period of 2.5 years (range 6 months to 10 years) by 38.48% (-5.47 to 86.67) (*P*=0.0003) in comparison to the baseline. Spleen size in the non-splenectomised patients showed a mean (range) decrease of 34.75% (0 to 91.67) (*P*=0.004).

Bone manifestations: Ten patients showed Erlenmeyer flask deformity of the femur. 9 had osteopenia and one had osteoporosis. One patient also developed avascular necrosis of the femur head and underwent osteotomy for abduction deformity. There was a subjective improvement in bone pains in all those who received ERT.

Neurological manifestations: Neurological manifestations were seen in 6 patients (Type III GD). There was ophthalmoplegia in 5 patients, occulomotor apraxia in 1 patient, retroflexion of the neck in 1 patient and difficulty in swallowing in 1 patient. The patients with neck retroflexion and swallowing difficulty showed improvement and the other neurological symptoms remained static and did not progress further. None of the patients had mental retardation or any history of seizures.

Mortality

Three patients (not included in the 22 analyzed patients) succumbed to the illness despite ERT. The first patient with type 1 GD, had presented with growth retardation and recurrent epistaxis since 3 years of age. At 9 years of age GD was diagnosed and splenectomy was performed. A year later, he had a variceal bleed, which was treated endoscopically. He was also diagnosed to have hepato-pulmonary syndrome with clubbing and cyanosis, which was

TABLE I CHANGE IN HEMOGLOBIN AND PLATELETS WITH ENZYME THERAPY

	Hemoglobin (g/dL) mean (range)	Platelets (10^2/L) mean (range)
6 months post ERT (*n*=22)	1.5 (3.4, 6.1) (*P*=0.01)	32 (-98.5, 145.5) (*P*=0.02)
1 year post ERT (*n*=8)	2.2 (-0.6, 6.4) (*P*=0.002)	133 (-22, 313) (*P*=0.003)

**ERT- Enzyme replacement therapy.*

TABLE II CHANGE IN WEIGHT AND HEIGHT WITH ENZYME THERAPY

	Weight (kgs) Mean (range)	Height (cm) Mean (range)
6 months post ERT (*n*=22)	3 (-5.6 to 10.5) (*P*=0.04)	7.1 (0 to 26.5) (*P*=0.0003)
1 year post ERT (*n*=8)	5.3 (-4.4 to 23.5) (*P*=0.02)	8 (1 to 32.5) (*P*=0.0004)

**ERT- Enzyme replacement therapy.*

proven on a Tc 99m macroaggregated albumin scan. He was started on ERT. During the treatment over one and a half years, he showed remarkable improvement with his hemoglobin increasing from 8.8g/dL, his platelet counts increased from 34×10^9/L to 230×10^9/L and serum albumin increased from 1.4 g/dL to 3.5 g/dL. His height increased by 10 cm and weight by 5 kg. After one and half years of being on treatment, he succumbed to respiratory distress after developing pneumonia. This was as unusual as patients with good response on ERT do well in the long term.

The second patient had type III GD and had presented with anemia and abdominal distension due to massive hepatosplenomegaly. She was dependent on blood transfusions for severe anemia. Her hemoglobin was 4.7 g/dL and platelets of 80,000/cmm. She was initially diagnosed as Type 1 GD. She received ERT for a period of 3 years. During the course of her treatment, her hemoglobin increased to 9.5 g/dL, platelets increased to 430×10^9/L. Her height increased by 10 cm and weight by 1.96 kg. Three months prior to her death, her hemoglobin fell to 5 g/dL and the hepatosplenomegaly increased. Ultrasonography of the abdomen revealed enlarged abdominal lymph nodes and she was also found to have a lateral gaze palsy (when the diagnosis was revised to Type III GD). She died due to respiratory distress associated with bronchospasm, after 3 years of treatment. This patient is similar to the one described by Burrrow, *et al*. [12], who developed progressive mesenteric and mediastinal lymphadenopathy over 12 months, despite enzyme replacement therapy.

The third patient was enrolled at the age of 6 years with failure to thrive, abdominal distension secondary to severe hepatosplenomegaly, epistaxis and bone pains. His hemoglobin was 8.6 g/dL and platelets of 137×10^9/L. His parameters did not improve and after a period of 1 and half years on ERT, he developed acute respiratory infection and breathlessness and died.

Adverse effects: One patient developed fever and breathlessness after the first enzyme infusion, but did not have the any further adverse effects when the infusions were given at a slower rate. None of the patients were administered any pre-medications.

DISCUSSION

Gaucher disease is believed to be extremely rare in India; however, its prevalence is not known. It is very likely that the prevalence is severely underestimated in part due to lack of access to diagnostic testing as well as a lack of awareness. In the majority of our patients (17/22), the diagnosis was not initially suspected but only made after either splenectomy or bone marrow biopsy. Unfortunately, very few centers in India have the facility to perform leukocyte acid glucosidase assay. Prior to the large series reported herein, there have been isolated case reports of GD from India [8-11] with only one patient receiving enzyme replacement therapy [8]. With the availability of leukocyte acid glucosidase testing and increasing awareness of the disease, we are beginning to diagnose Gaucher disease with an increasing frequency. Fifty percent of our patients had history of consanguinity. Also, the availability of enzyme therapy through the India Charitable Access Program of Genzyme Corporation, USA should stimulate clinicians to consider GD in the differential diagnosis in order not to miss this treatable lysosomal storage disorder.

This article presents the largest experience of enzyme replacement therapy in Gaucher disease in India. We document clinical response in terms of improvements in height, weight, hemoglobin, platelets, spleen and liver volumes. There was excellent response in all disease domains, including improvement of hematological parameters, reversal of organomegaly and improvement of growth parameters. Several patients were blood transfusion dependent and with ERT, they did not require further transfusions. One patient with advanced liver disease associated with hepatopulmonary syndrome showed a poor response and eventually succumbed. This underscores the importance of early diagnosis before irreversible complications of Gaucher disease occur.

Before the introduction of enzyme therapy [13], treatment for Gaucher disease was supportive, with splenectomy, blood transfusions, analgesics and orthopedic surgeries for bone pain. In selected patients, cure was possible with bone marrow transplantation, but the risk of the transplantation are high [14,15]. Enzyme replacement therapy has

WHAT IS ALREADY KNOWN?

- Gaucher disease is treatable with enzyme replacement therapy but diagnosis and treatment was not possible or affordable for most patients with the disease in India.

WHAT THIS STUDY ADDS?

- Imiglucerase enzyme replacement therapy showed satisfactory outcomes in majority of the treated children.

transformed the management of Gaucher disease. Lifelong therapy is recommended unless alternative therapies like gene therapy become available in the future.

Side effects related to Imiglucerase injection administration have been reported in less than 15% of patients. Reported side effects include nausea, vomiting, abdominal pain, diarrhea, rash, fatigue, headache, fever, dizziness, chills, backache, and rapid heart rate. At the site of injection, discomfort, itching, burning swelling or uninfected abscess can occur. Approximately 15% of patients have developed antibodies though all patients tolerate treatment [17]. Antibody levels were not monitored in our patients. One of our patients had side effects, during the first injection, which did not recur again. It could also have been a pyrogenic reaction.

Andersson, *et al*. [18], in a large series of 884 patients, documented normalization of hemoglobin, height and weight over a period of 8 years and significant improvement in the first 2 years. The liver and spleen volumes also reduced significantly within the first 2 years. Though we do not have comparative long term data, our medium term follow up of 1 year in the Indian patients revealed that the hemoglobin increased by 2.2g/dL, 50% of patients achieved normal platelet counts and the liver and spleen volumes decreased by about 1/3rd the original volumes over a mean period of 2.5 years.

This article should help improve awareness of GD when a patient presents with organomegaly and/or cytopenia and/or bone pain. This cohort of GD patients reported excellent outcomes of imiglucerase enzyme replacement therapy.

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REFERENCES

1. Grabowski GA, Gatt S, Horowitz M. Acid beta-acid beta glucosidase: Enzymology and molecular biology of Gaucher disease. Crit Rev Biochem Mol Biol. 1990; 25: 385-414.
2. Kaplan P, Andersson HC, Kacena KA, Yee JD. The clinical and demographic characteristics of nonneuronopathic Gaucher disease in 887 children at diagnosis. Arch Pediatr Adolesc Med.2006;160:603-8.
3. Kauli R, Zaizov R, Lazar L, Pertzelan A, Laron Z, Galatzer A, *et al.* Delayed growth and puberty in patients with Gaucher disease type 1: natural history and effect of splenectomy and/or enzyme replacement therapy. Isr Med Assoc J. 2000;2:158-63.
4. Grabowski GA, Andria G Baldellou A, Campbell PE, Charrow J, Cohen IJ, *et al.* Pediatric non-neuronopathic Gaucher disease: presentation, diagnosis and assessment: consensus statements. Eur J Pediatr.2004;163:58-66.
5. Hopkin RJ, Grabowski GA. Lysosomal storage disease. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, *et al.* (eds). Harrison's Principles of Internal Medicine, 17th Edition: 2008.
6. El-Beshlawy A, Ragab L, Youssry I, Yakout K, El-Kiki H, Eid K, *et al.* Enzyme replacement therapy and bony changes in Egyptian paediatric Gaucher disease patients. J Inherit Metab Dis.2006;29:92-8.
7. Ginns EI, Erickson A, Tegelaers FP, Barneveld R, Reuser AJ, Brady RO, *et al*. Isozymes of beta-acid beta glucosidase: determination of Gaucher's disease phenotypes. Isozymes Curr Top Biol Med Res. 1983;11: 83-9.

8. Kasturi L, Amin AS. Enzyme replacement therapy in Gaucher disease. Indian Pediatr.2001;38:686-8.
9. Kaur M, Kabra M, Kher A, Naik G, Bharucha BA, Verma IC. Clinical and enzyme studies in Gaucher disease. Indian Pediatr.1996;33:735-8.
10. Feroze M, Arvindan KP, Jose L. Gaucher disease among Mappila Muslims of Malabar. Indian J Pathol Microbiol 1994;37:307-11.
11. Patel AL, Shaikh WA, Khobragade AK, Soni HG, Joshi AS, Sahasrabudhe GS, *et al*. Gaucher disease. J Assoc Phys India.2009;57;410-11.
12. Burrow TA, Cohen MB, Bokulic R, Deutsch G, Choudhary A, Falcone RA Jr, *et al*. Gaucher disease: progressive mesenteric and mediastinal lymphadenopathy despite enzyme therapy. J Pediatr.2007;150:202-6.
13. Grabowski GA, Barton NW, Pastores G. Dambrosia JM, Banerjee TK, McKee MA, *et al.* Enzyme therapy in type 1 Gaucher disease : comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. Ann Intern Med.1995;122:33-9.
14. Tsai P, Lipton JM, Sahdev I, Najfeld V, Rankin LR, Slyper AH, *et al.* Allogenic bone marrow transplantation in severe Gaucher disease. Pediatr Res.1992;31: 503-7.
15. Hobbs JR, Jones KH, Shah PJ, Lindsay I, Hancock M. Beneficial effect of pre-transplant splenectomy on displacement bone marrow transplantation for Gaucher's syndrome. Lancet.1987;1:1111-15.
16. Pastores GM, Sibille AR, Grabowski GA. Enzyme therapy in Gaucher disease type 1: dosage efficacy and adverse effects in 33 patients treated for 6 to 24 months. Blood. 1993;82:408-16.
17. Starzyk K, Richards S. Yee J, Smith SE, Kingma W. The long-term international safety experience of imiglucerase therapy for Gaucher disease. Mol Genet Metab.2007;90: 157-63.
18. Andersson H, Kaplan P, Kacena K, Yee J. Eight-year clinical outcomes of long-term enzyme replacement therapy for 884 children with Gaucher disease type 1. Pediatrics.2008;122;1182-90.