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Outcome of enzyme replacement therapy in children with Gaucher disease: The Egyptian experience

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Abstract Gaucher disease is the most prevalent lysosomal storage diseases which results from inherited deficiency in the glucocerebrosidase enzyme. Three main clinical forms have been described: type I non-neuropathic, type II acute neuropathic and type III subacute neuropathic. Although it is panethnic disease, its presentation has some ethnic specific characteristics. In this work, we present specific characteristics as well as our experience in diagnosing and managing a group of Egyptian patients with this disease. The study included 48 patients with Gaucher disease attending Children’s Hospital, Ain Shams University. The recombinant enzyme imiglucerase (cerezyme) was given in a dose of 60 U/kg/2 weeks. Haemoglobin, platelet count, plasma chitotriosidase, and abdominal ultrasound were assessed before starting therapy and every 6 months. Molecular analysis was done to 23 patients. At presentation, the mean age was 2.54 ± 3.8 years. Ten patients (20.8%) had type I, 6 had type II (12.5%) and 26 had type III Gaucher disease
1. Introduction

Gaucher disease (GD) is the most frequently encountered lysosomal storage disease caused by inborn defects of the membrane-bound lysosomal enzyme, acid β-glucosidase or glucocerebrosidase. This defective activity causes an accumulation of glucocerebroside in the lysosomes of cells derived from the monocyte/macrophage lineage. Glucocerebrosidase-engorged cells, termed Gaucher cells, infiltrate various organs, leading to multisystem abnormalities [1]. GD is classified into three main types based according to the presence and nature of CNS involvement; type II (infantile type) has infantile onset of severe CNS involvement and death in early childhood; and type III has onset of mild CNS involvement in adolescence or early childhood and has a more indolent course. The most common form is type I (adult type), the age of onset and rate of progression varies widely, ranging from disability in the common form is type I (adult type), the age of onset and rate of progression varies widely, ranging from disability in toddler to asymptomatic disease [2]. An atypical form of GD (cardiovascular form) dominated by cardiovascular involvement with calcification of the mitral and aortic valves has been described by Bohlegha et al. [3]. Additional manifestations may include mild splenomegaly, corneal opacities, and supranuclear ophthalmoplegia [4]. A perinatal lethal form associated with hepatosplenomegaly, pancytopenia that may present clinically as ichthyosiform or collodion skin abnormalities or as non-immune hydrops fetalis has been described by Orvisky et al. [5]. Another rare severe variant of GD associated with hydrocephalus, corneal opacities, deformed toes, gastrointestinal reflux, and fibrous thickening of splenic and hepatic capsules was described by Inui et al. [6].

In this study, we present the experience in diagnosis and management of 48 Egyptian children with GD, attending the Children’s Hospital, Ain Shams University.

2. Patients and methods

The study included all patients with GD (48) diagnosed from June 1995 to December 2006 in the Haematology and Genetics Clinics, Children’s Hospital, Ain Shams University which is the second largest university in Egypt and a tertiary referral center.

2.1. Diagnosis

For all patients the following was done before starting therapy and then every 6 months after starting therapy:

- Full history, three generation family pedigree construction and thorough clinical examination laying stress on growth parameters, pallor, bleeding manifestation, hepatosplenomegaly and full cardiac and neurological examination.
- Laboratory investigations included complete blood count (CBC) and reticulocyte count (anaemia and thrombocytopenia were defined according to the age and sex specific values), prothrombin time (PT) and partial thromboplastin time (PTT), liver function tests, renal function tests, bone marrow aspirate (done only before starting therapy), liver biopsy and/or splenic aspirate if needed.
- Radiological investigations included plain X-ray for long bones and chest, abdominal ultrasonography to evaluate liver and spleen volumes, echocardiography, DEXA scan and MRI of femurs for selected patients.
- Measurement of plasma chitotriosidase and assessment of β-glucosidase enzyme activity in peripheral leucocytes. The chitotriosidase normal level ranges from 4 to 80 μmol/L/h [7]. Normal enzyme level ranges from 1 to 5 μmol/h/g protein [8].
- Analysis of common mutations was performed for 23 patients by PCR and full sequencing of GBA gene [9].
- Family screening was done in five families by measuring β-glucosidase enzyme activity in peripheral leucocytes. GD was detected in five sibs including two from one family.
- Prenatal diagnosis was done in two families and fetuses proved to be normal.

2.2. Enzyme replacement therapy (ERT)

ERT started in 1997 using the placenta derived aglucerase (ceredase) at low dose regimen (15 U/kg/2 weeks). Treatment with the recombinant imiglucerase (cerezyme) began on March 1999 in a dose of 20–30 U/kg/month, divided into four equal weekly doses. In this period, ERT was started in 17 patients and discontinued in three patients: The first patient was 3.5 years old and had advanced type I GD. He died 4 weeks after the beginning of ceredase therapy of nephrotic syndrome and progressive renal failure. The second patient had advanced type III GD and died 2 months later with extensive bronchopneumonia. The third developed chronic myeloid leukemia after 6 months of good response to ERT. Since the first of July 1999, Cerezyme was administered in a high dose regimen of 60 U/kg/2 weeks for 48 patients. The protocol was approved by Gaucher registry and informed consent was obtained from the parents of all patients.
2.3. Statistical methods

Standard computer program SPSS for Windows, release 13.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was done using Student's t-test and Mann–Whitney test for normal and non-parametric variables, respectively. Multiple regression analysis was also performed to determine effect of various factors on a dependent variable. For all tests a probability (p) less than 0.05 was considered significant [10].

3. Results of patients on ERT

Patients’ ages at presentation ranged from 37 days to 16 years with mean age of 2.54 ± 3.8 years. Male to female ratio was 3.5:1. Consanguinity between parents was present in 43 patients (88.8%). This included first cousin’s marriage in 32 cases (74.4% of consanguineous marriage), second cousins’ marriage in five cases (11.6%) and remote consanguinity in six cases (13.9%). Family history was positive in 26 patients (54.16%).

Bone marrow aspiration was done to all patients and revealed lipid engorged macrophages (Gaucher cells) in 40 of patients (83.3%). Liver biopsy was done in two patients and splenic aspirate was done to five patients. All revealed typical Gaucher cells. Splenectomy was done in three patients because of hypersplenism before starting ERT therapy.

Plasma chitotriosidase activity was markedly increased in all patients with mean of 1612.6 ± 2116.54–80 μmol/L/h. Diagnosis was confirmed by assessment of β-glucosidase enzyme activity in peripheral leucocytes which was decreased to 0–15% of normal values in all patients. It was the only diagnostic tool in nearly 17% of patients (in conditions when Gaucher cells could not be detected in bone marrow and during screening of families of patients).

Initially, 29 patients had clinically type I GD (60.4%). With follow up, neurological manifestations started to appear in 19 children and so they were re-categorized as type III GD. Also two patients who were initially diagnosed as having type III GD, proved later to have type II GD. Final diagnosis included 10 patients with type I GD (20.8%), 6 patients with type II GD (12.5%) and 32 patients with type III GD (66.67%) (Fig. 1).

Following up the patients receiving ERT for 24 months revealed a significant increase in weight after 6 months of ERT (p = 0.045) that was progressed after 12 months (p = 0.01), 18 months (p = 0.014) and not after 24 months (p = 0.084). On the other hand, patients’ heights were gradually increasing although statistically not significant. At presentation haemoglobin level (Hb) varied between 5 and 11 g/dL (mean 8.3 ± 1.5 g/dL). There was a significant increase in Hb level after ERT with no significant difference between types I and III. It is to be noted that GD patients usually become transfusion independent after 2–3 doses of enzyme therapy. This is unlike platelets count which was significantly increased only after 24 months of ERT (Table 1).

Plasma chitotriosidase levels showed significant decrease after 6 and 12 months of ERT assay with no significant difference between types I and III GD. After 24 months its mean level was 288 ± 309.7 μmol/L/h (Table 2). There was slow reduction in ultrasound measures of liver span during the 24 months follow up period with no significant difference between types I and III except in two patients who developed progressive hepatomegaly as they developed viral hepatitis type C. On the other hand, ultrasound splenic length showed significant reduction in the 24 months follow up period with no significant difference between types I and III GD. The response to ERT was variable in different patients even in the same phenotype in both the liver span and splenic length.

While there was mild improvement in motor development and variable improvement of dysphagia (although at slower rate than visceral improvement) on ERT, ophthalmoplegia did not show significant improvement in type III patients. Individual variations were also observed in patients’ response to ERT regarding neurological manifestations. Bone manifestations showed very slow improvement on ERT that needed more time to become evident but generally, all patients demonstrated improvement in quality of life in an average of 6 months period.

Respiratory complications were present in seven patients (14.6%). Six of them had moderate pulmonary infiltrate with recurrent attacks of respiratory distress and wheezy chest and one patient developed extensive pulmonary infiltrate that needed recurrent ICU admission (type III GD, genotype L444P/L444P) (Fig. 2). Renal manifestations were present in two patients (5.56%), one had kidney stones (type III GD) and the other had nephrotic syndrome (type I GD). Cardiac

![Figure 1](image)

**Figure 1** Patient distribution according to the diagnosis at enrollment and final diagnosis.
manifestations were present in six patients. Concentric left ventricular hypertrophy and systemic hypertension was present in one patient with type III GD. Two patients had pericardial effusion and cardiomyopathy (one type I and one type III). Calcification of aortic and mitral valves and hypertrophy of interventricular septum were present in one patient cardiovascular form (genotype: D409H/D409H) (Fig. 3). The last patient’s younger brother had dilatation of both ventricles, thickened right and left coronaries, thickening of the aortic valve and tricuspid regurgite. His younger sister had dilatation of ventricles, mild pulmonary hypertension and early myopathy.

We tried to reduce the ERT dose from 60 to 45 U/kg/2 weeks after 3 years of good response to therapy in four patients: the first was a 16 year old girl with type I GD (N370S/N370S) but she developed bone pains and osteoporosis after 6 months of dose reduction and therefore bisphosphonates was added. The second was a 7 year old boy with cardiovascular form (D409H/D409H) (Fig. 3). The last patient’s younger brother had dilatation of both ventricles, thickened right and left coronaries, thickening of the aortic valve and tricuspid regurgite. His younger sister had dilatation of ventricles, mild pulmonary hypertension and early myopathy.

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On the other hand, we tried to increase ERT dose from 60 to 120 U/kg/2 weeks in two patients: the first was a 2.8 year old boy with type III (L444P/L444P) with extensive pulmonary infiltrates, concentric left ventricular hypertrophy and recurrent admission to ICU. An improvement was noticed for few months but after 9 months he died suddenly at home during his sleep with unidentified cause. The second is a 7 year old boy with type III (L444P/L444P) who was receiving ERT since the age of 20 months and developed progressive neurological manifestation and myoclonic fits. The convulsions were controlled after dose increase.

ERT was discontinued in three patients: the first is a 4.5 year boy with type I (P266L/N370S) who had no response after 1 year and his father decided to discontinue. The second is a 6 year old boy who was initially diagnosed as type I GD and had no response after 1 year of regular ERT. Reevaluation revealed that he has Niemann-Pick type C disease. The third is a 9 month old girl with type III GD who had no response after 1 year with progressive hepatosplenomegaly and neurological manifestation. Reevaluation revealed that she had type II GD.

### 4. Discussion

In this study, most of our patients were of type III GD (66.67%), which is different from what was found by the Inter-
national Collaborative Gaucher group in which most patients were of type I GD [11]. This may be due to inclusion of only children with GD and not inclusion of adult group. The reclassification of 19 patients of type I into type III emphasizes the importance of careful follow up of neurological symptoms in this population (especially that type III appears to be the most common type) and indicates that the dose of 60 U/kg/2 weeks do not prevent the progression of neurological disease although it might delay its appearance.

Anemia in GD may result from haemolysis secondary to hypersplenism, haemodilution, and splenic sequestration, and in more advanced cases (particularly post-splenectomy), from depressed erythropoiesis associated with Gaucher cells infiltration of the bone marrow. Thrombocytopenia occurs as a result of hypersplenism, splenic pooling of platelets, and/or marrow infiltration [12]. Improvement in hematologic cytopenias is among the earliest and most sensitive indicators of response to ERT [13]. In our patients, haemoglobin level varied between 5 and 11 g/dL (mean 8.3 ± 1.5 g/dL) at presentation. It increased to normal or near normal within 6–12 months with a significant difference between types I and III GD patients. Patients usually become transfusion independent after 2–3 doses of enzyme therapy. The same was true for platelet count. The same observations were documented by the international Gaucher registry [14].

The most common mutation found in this study was L444P/L444P which was detected in 56.5% of patients while N370S/N370S represented only 13.04% of mutations detected. This is consistent with what was found in non-Jewish population, as the most common mutations were L444P, N370S, D409H, R463C and IVS+1 [15] and different from what was reported by the International Collaborative Gaucher group where N370S/N370S was the most common detected mutation (29%) and L444P/L444P accounted for only 6% for detected mutations [16]. This is because most of our patients were of type III GD in which homozygosity for the L444P mutation was the most common genotype [17].

GD is characterized by considerable variability in its clinical signs and symptoms. This phenotypic variability is observed, even amongst siblings with the same genotype [18]. There is also significant lack of understanding of how the expression of the phenotype is modified by epigenetic and environmental factors to affect disease expression and severity. Some correlation has been observed between genotype and phenotype in GD. However, prediction of organ involvement, disease severity and rate of progression based on mutation analysis is generally not possible, due to the extremely wide variation in disease expression, even among specific ethnic groups [17]. In the present study, there was no clear association between genotype and phenotype. ERT response was also variable even in same phenotype and genotype. It was evident that genotype N370S is present in all patients with type I GD either in the homozygous (50%) or heterozygous form (50%). Its presence usually ameliorates the clinical presentation and protects against the development of neurologic disease. On the other hand, homozygote L444P was present in all patients with type III disease. This is consistent with Grabowski study who suggested that the homozygous N370S allele is usually associated with a generally less severe phenotype, although with wide clinical variability. The heterozygous state for N370S is protective against CNS involvement; and the L444P allele in the homozygous state is associated with early neurologic symptoms common in types II and III [19]. However in this study, two children with homozygous L444P mutation (8.6%) exhibited type I phenotype and they need careful observation as they may develop neuropathic disease as they grow older. The same was also previously reported in another Egyptian study [20].

Pulmonary involvement reported in GD includes interstitial lung disease; alveolar/lobar consolidation; and pulmonary hypertension [21,22]. In this study, respiratory complications were present in seven patients (14.6%). Increasing the dose to 120 U/kg/2 weeks showed initial improvement. On the other hand, fractionation of the dose to 60 U/kg/week had better effect on one patient. Unfortunately follow up of this patient could not be done as he died suddenly during his sleep few months later.

We had one family in this study with three children with the cardiovascular form (homozygous D409H). The older brother had calcification of the mitral and aortic valves, the younger brother had dilatation of both ventricles, thickened right and left coronaries, thickening of the aortic valve and tricuspid regurgite while the youngest sister had dilatation of ventricles, mild pulmonary hypertension and early myopathy. This again demonstrates the variability of clinical presentation of the same genotype. Cardiomyopathy and pericardial effusion was found in two of our patients (one type I and one type III). Reports of myocardial involvement in GD included decreased left ventricular compliance with decreased cardiac output [23], wide spread acute haemorrhagic necrosis of the left ventricular myocardium with fibrosis of coronary arteries and left ventricular hypertrophy [24]. The effect of ERT on this problem still needs follow up.

In our cohort, we had two other patients with renal problems; the first had type I with nephritic syndrome in which ERT did not stop the progression of his condition into renal failure. The other had type III GD (L444P/L444P) with multiple renal stones. Reports of nephritic syndrome in GD are few with a well defined glomerulopathy often with Gaucher cells in the glomeruli [25]. Other reports included developmental defects of the urinary system with bilateral megaureter and hydronephrosis, developmental defects of the renal arteries, and bilateral cystic renal dysplasia [26]. To the best of our knowledge, kidney stones were not reported in GD before.

Severe bone disease was observed in two patients (in the form of Erlenmeyer flask shape deformity, bone fractures and bone pains) and mild bone disease (bone aches) in one patient. They had dramatic improvement in the form of decreasing bone aches and cessation of fractures but follow up is essential. Although increased bone turnover rates are not proven, anti-osteoclastic therapy, primarily with bisphosphonates, calcium and vitamin D has been suggested as an adjunctive therapy for skeletal manifestations [27]. Treatment with bisphosphonates in the form of bonapex in a dose of 10 mg/day was given as the only treatment in one patient with GD type I and as adjuvant treatment to another two patients with type I GD. All three patients had splenectomy before diagnosis. However, in another Egyptian study ERT was effective in ameliorating radiological manifestations of skeletal disease and achieving complete remission of bone pains after 11.2 ± 4 months [20].

The safety profile of imiglucerase is excellent. Only two patients developed allergic reactions with no antibodies detected. The same was reported by Elstein and Zimran [28].
5. Conclusion

ERT with imiglucerase could improve the quality of life in patients with GD by ameliorating the GD associated anaemia, thrombocytopenia, organomegaly, growth retardation and bone pain. Since most Egyptian children with GD has type III disease and L444P/L444P genotype, a minimum dose of 60 U/kg/2 weeks should be maintained until adulthood. Dose reductions may cause early appearance of previously absent neurological symptoms. Higher doses started at an early age may delay the progression of neurological symptoms. Starting dose of 90–120 U/kg/2 weeks particularly for younger patients with normal intellect may be recommended.

Clinical variability exists in patients carrying the same genotype and between members of the same family. Pulmonary involvement is not rare in Egyptian patients and may respond to dose increase or dose fractionation. CVS and renal symptoms should be further studied in our population.

6. Disclosure statement

We declare that there are no conflicts of interests.

References