



Revista Brasileira de Hematologia e Hemoterapia Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org



Original article

Study of enzyme replacement therapy for Gaucher Disease: comparative analysis of clinical and laboratory parameters at diagnosis and after two, five and ten years of treatment

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ARTICLE INFO

Article history:

Received 7 September 2013

Accepted 17 March 2014

Available online 28 May 2014

Keywords:

Gaucher disease

Enzyme replacement therapy

Anemia

Splenomegaly

ABSTRACT

Objective: To evaluate the impact of enzyme replacement therapy for Gaucher Disease on clinical and laboratory parameters after two, five and ten years of treatment.

Methods: Data were collected from patient records and analyzed using BioEstat software (version 5.0). Student's t-test, Analysis of Variance (ANOVA), Wilcoxon test and Kruskal–Wallis test were used for statistical analysis. Hepatomegaly and splenomegaly were analyzed using the Kappa test.

Results: There was a significant increase in hemoglobin levels (p -value <0.01) and platelet counts (p -value $=0.01$) within two years of therapy. At the same time, the frequencies of splenomegaly (p -value <0.01) and hepatomegaly (p -value <0.05) reduced. These results were similar at five and ten years of enzyme replacement therapy.

Conclusions: There are substantial and quick (within two years) laboratory and clinical responses to enzyme replacement therapy. These improvements continue as long as enzyme replacement therapy is administered every two weeks, as recommended by the literature.

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Introduction

Gaucher Disease (GD) is a recessive autosomal hereditary disorder classified as an inborn error of the metabolism. It is the commonest lysosomal storage disease and was the first one for which a specific treatment was developed. It occurs due to

a deficiency in the activity of the enzyme β -glucosidase and is characterized by the intra-lysosomal accumulation of glucocerebroside in reticuloendothelial system cells.¹ The enzyme deficiency is caused by a mutation in the β -glucosidase gene, located on chromosome 1 (GBA1).² GD is a rare pan-ethnic disorder, but it presents a high incidence among Ashkenazi Jews. The worldwide incidence is estimated at from 1:50,000

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<http://dx.doi.org/10.1016/j.bjhh.2014.05.005>

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to 1:100,000 live newborns,³ whereas the incidence in the Jewish population ranges from 1:400 to 1000 live newborns in the USA.⁴

Clinically, GD presents a wide variety of signs and symptoms and is classified as non-neuronopathic (Type 1) or neuronopathic (Types 2 and 3). Type 1 GD (95% of cases) usually manifests with splenomegaly, hepatomegaly, anemia, thrombocytopenia, bone disease and delayed growth.⁵ Type 2 is characterized by a precocious and fast brainstem degeneration;³ these patients do not respond to treatment and death occurs within the first two years of life.⁶ Type 3 GD patients have a slow evolving neurologic disease and usually present with seizures, eye movement abnormalities and mild systemic involvement with mean survival being to the third decade of life.⁷ The treatment of GD is based on enzyme replacement therapy (ERT), initially by alglucerase,⁸ but this was widely substituted by its therapeutic equivalent, imiglucerase.⁹ Accordingly to Brazilian Ministry of Health, there were 610 ERT-dependent patients in the country in 2010. There are no data concerning the national incidence of the disease.¹⁰

The objective of this study was to evaluate the impact of ERT on clinical and laboratory parameters of GD through a comparative analysis of data at diagnosis and after two, five and ten years of treatment in a population from Pará State, Brazil.

Methods

This was an analytical observational longitudinal retrospective study (historical cohort) of patients at the Fundação Centro de Hemoterapia e Hematologia do Pará (HEMOPA), Belém, Pará State. The patients were diagnosed with non-neuronopathic (Type 1) and neuronopathic (Type 3) GD and were treated and followed-up at HEMOPA between 2000 and 2011.

The inclusion criteria for the study were to have a confirmed diagnosis of GD and to be treated and followed-up at HEMOPA for at least 24 months. Clinical records prior to treatment were also necessary.

Data were collected from patient records using a questionnaire designed for this study. This questionnaire included the following items: demographic characteristics, genetic profile, ERT dose, hematological aspects (hemoglobin levels, white cell count and platelet count) and clinical manifestations (splenomegaly, hepatomegaly and neurological symptoms). Data were collected at four different time points: at diagnosis and after two, five and ten years of ERT. Diagnosis was defined as the time when Gaucher's cells and/or the β -glucosidase deficiency were identified. Data concerning the two-, five- and ten-year time points were collected based on the first administration of ERT. This study was approved by the Ethics Committee of HEMOPA (register # 0016.0.324.324-11).

Statistical analysis

Data were placed in tables and graphs drawn using the Microsoft Excel 2010 software. ERT doses, hemoglobin levels, white cell count and platelet count over time were analyzed

using Student's t-test, Analysis of Variance (ANOVA), Wilcoxon and Kruskal-Wallis tests, as applicable. Splenomegaly and hepatomegaly were analyzed using the Kappa test. All results with p -values <0.05 were considered significant and tests were carried out using the BioEstat (version 5.0) software.

Results

Demographic characteristics

Records of 24 patients diagnosed with GD were found and of these, 13 met the inclusion criteria. Eight were female (61.50%) and five were male patients (38.50%). Ages ranged from four to 43 years (mean: 24.53 years). Thirteen patients (100%) were on ERT for two years; nine (69.23%) had completed five years of treatment and six patients (46.15%) had been treated for ten years. In order to preserve patients' identity, they are numbered 1 through 13.

At diagnosis, the mean age of patients was 13.49 years old (± 30.10 years). Eight patients (61.53%) were under 12 years old at diagnosis (patients 1, 3, 4, 5, 8, 11, 12 and 13), three (23.07%) were women over 12 years (patients 6, 9 and 10) and two (15.4%) were men over 12 years (patients 2 and 7). Only one patient (7.7%) was diagnosed with Type 3 GD; this patient died at age 22 after ten years of treatment. All other patients were diagnosed as Type 1.

Genetic profile

Ten patients (76.92%) were submitted to GBA1 mutation analysis with the frequencies of the mutations listed in Fig. 1.

ERT dose

ERT was administered every two weeks. Table 1 shows the amount of enzyme (IU/kg) given to each patient during the study intervals.

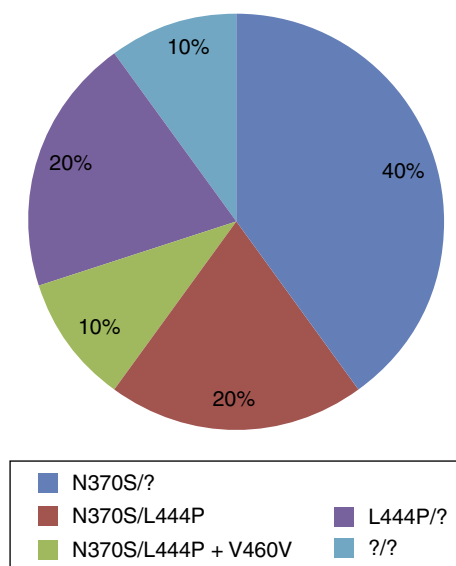


Figure 1 – Frequency of mutations in patients with GD submitted to GBA1 analysis at HEMOPA.

Table 1 – Enzyme replacement therapy dose administered (IU/kg) to patients with Gaucher Disease by study interval.

Patient	At start	2 years	5 years	10 years
1	27.9	25.0	28.0	30.0
2	30.0	30.0	26.0	–
3	36.6	40.0	–	–
4	30.0	30.0	–	–
5	18.4	42.1	–	–
6	33.3	37.1	30.0	15.0
7	44.0	44.0	41.0	37.0
8	22.0	61.0	54.0	–
9	35.0	24.6	27.0	15.0
10	15.0	29.0	29.0	–
11	37.7	37.7	26.9	15.0
12	32.4	40.0	–	–
13	29.4	13.6	40.0	85.7

There was no statistical difference in the dose received between any of the study intervals (p -value = 0.78). Mean dose of all periods together was 32.88 IU/kg.

Laboratory parameters

Table 2 presents hemoglobin levels (g/dL) at diagnosis and after two, five and ten years of treatment.

The mean hemoglobin level at diagnosis (9.9 g/dL) and after two years of treatment (12.4 g/dL) were compared using Student's t -test. The mean difference between these two periods (2.5 g/dL) was statistically significant (p -value = 0.0039; 95% confidence interval: 0.0762–4.15). ANOVA, however, did not show statistical difference when the intervals of 2, 5 and 10 years were compared. On the other hand, hemoglobin levels at 5 and 10 years were significantly higher than the levels at diagnosis (p -value < 0.01 for both periods).

Table 3 shows the total white cell count in the different study intervals.

No statistical significance was found for the white cell count between any of the study intervals.

Table 4 presents the platelet counts at diagnosis and after two, five and ten years of ERT.

Table 3 – White cell count ($\times 10^9/L$) of patients under enzyme replacement therapy for Gaucher Disease by study interval.

Patients	At diagnosis	2 years	5 years	10 years
1	4.20	3.40	3.13	4.35
2	10.60	9.93	9.58	–
3	1.80	1.06	–	–
4	4.56	6.30	–	–
5	6.71	5.66	–	–
6	11.30	11.70	13.80	13.10
7	5.50	15.40	10.80	10.70
8	12.30	17.80	6.91	–
9	4.40	5.30	4.81	7.00
10	8.80	5.80	6.50	–
11	3.00	2.90	3.88	4.23
12	6.18	9.30	–	–
13	2.90	3.00	3.94	3.00

The difference in platelet counts between diagnosis and after two years of treatment, evaluated using the Wilcoxon test (non-normal distribution at diagnosis), was statistically significant (p -value = 0.01). The differences between the other periods were not statistically significant.

Clinical manifestations

On analyzing splenomegaly, two patients were excluded because they had been submitted to splenectomy prior to ERT. All of the 11 remaining patients presented splenomegaly at diagnosis, detected either by physical exam or radiological imaging. After two years of ERT, 63.63% of the cases presented with splenomegaly. This reduction was statistically significant (p -value < 0.05). A reduction, albeit non-significant, was found in the frequency of splenomegaly comparing patients after only two years of treatment and after five and ten years of treatment.

Similar results were found concerning hepatomegaly. Of the 13 patients, 69.23% had hepatomegaly at diagnosis. At the end of two years of ERT, only 30.77% continued with any degree of hepatomegaly. This reduction was significant (p -value < 0.05). There was a further reduction in the number of

Table 2 – Hemoglobin levels (g/dL) of patients under enzyme replacement therapy for Gaucher Disease by study interval.

Patient	At diagnosis	2 years	5 years	10 years
1	10.0	14.0	11.4	13.4
2	8.2	16.1	13.9	–
3	10.5	9.6	–	–
4	12.2	11.8	–	–
5	12.6	13.8	–	–
6	10.6	12.0	10.5	12.2
7	12.8	12.2	13.4	13.9
8	9.5	11.9	11.3	–
9	8.2	13.6	13.8	14.0
10	9.4	10.0	12.3	–
11	7.4	13.8	13.6	14.6
12	8.7	10.7	–	–
13	9.0	11.4	12.5	10.5

Table 4 – Platelet count ($\times 10^9/L$) of patients under enzyme replacement therapy for Gaucher Disease by study interval.

Patients	At diagnosis	2 years	5 years	10 years
1	90.0	87.0	74.9	121.0
2	105.0	348.0	378.0	–
3	20.0	20.3	–	–
4	240.0	205.0	–	–
5	117.0	165.0	–	–
6	390.0	336.0	317.0	127.0
7	200.0	232.0	222.0	312.0
8	112.0	258.0	204.0	–
9	87.0	165.0	178.0	186.0
10	120.0	154.0	128.0	–
11	59.0	101.0	71.4	131.0
12	92.4	158.0	–	–
13	53.0	105.0	130.0	1560

patients with hepatomegaly at 5 and 10 years of treatment, but the differences were not statistically significant.

Neurological symptoms were noticed only in the Type 3 patient in the study sample. This patient presented seizures, tremors of the extremities, dyslalia and difficulties walking during all the period of ERT (10 years).

Discussion

GD is usually described as a pan-ethnic disease, attacking men and women in equal proportions. However, this study presented a higher prevalence of female (61.5%) compared to male patients (38.5%). This data is similar to a study by Sobreira & Bruniera,³ who showed a higher prevalence (67.8%) of female patients, and differs from Ferreira et al.,¹ who reported a slight predominance of male patients. This is attributed to the small sample size common when studying rare conditions. No data relating the patients to Ashkenazi parentage were found.

Mean age at diagnosis was slightly higher than that reported by Sobreira and Bruniera (11.8 years old)³ and lower than that in the study of Charrow et al. (17.4 years old).¹¹ Giraldo et al.¹² reported a mean age at diagnosis of 27.4 years old. Earlier manifestations of the disease are typical for Latin-American populations; in these patients, the first symptoms of GD occur at 11 years of age and can be more severe.¹³ Such differences show that GD can present at differing ages in different populations and is an expression of the disease's heterogeneity.

The N370S mutation, in 70% of the individuals submitted to GBA1 analysis, was the most common in this study sample. This data is similar to a study of 1698 patients from the Gaucher Registry,¹¹ in which 84% of individuals had at least one N370S allele; however, 23% were homozygous for this mutation, different to the current study, in which no homozygous individuals were found. The second most frequent mutation was L444P, which occurred in 50% of the sample. This frequency is slightly higher than that of Charrow et al. (30%),¹¹ Kaplan et al. (34%)⁵ and considerably higher than that of Andersson et al. (23.7%).¹⁴ Even so, no homozygous individuals with the L444P mutation were found in this sample. No other mutation that is considered common in the literature,⁶ such as 84GG and IVS+1, was found. On the other hand, the V460V mutation, which is not considered a common mutation, was associated to the L444P mutation.

The divergences between these data are most likely due to the fact that the aforementioned studies gathered information from five continents and that Latin-America represented a small portion of the total. A study of just Latin-American patient records in the Gaucher Registry¹³ found a low frequency of L444P homozygosis. In the same study, 82% of individuals had at least one N370S allele, but only a small portion of them was homozygous. A small study in Santa Catarina State, Brazil¹ corroborates these findings; 60% of individuals were heterozygous for the N370S mutation and there was only one homozygous case. The L444P mutation was found in 30% of individuals and there was one case of homozygosis. The differences between studies in Brazil, Latin-America and worldwide show that Brazilian and Latin-American patients have a genetic profile that differs from

that of patients from other nations with such differences being expressed as diverse phenotypic presentations of the disease.

Furthermore, a study with 48 Brazilian patients from different regions of the country found seven different previously unknown mutated sequences of GBA. Five were missense changes (S125N, F213L, P245T, W378C, D399H), one was an in-frame insertion and the other one was a splicing mutation in a complex allele (L461P + IVS10 + 1G > T). These data reinforce the genetic heterogeneity of Brazilian GD patients as was also found in the current research. This study also suggests that the presence of the N370S allele would be a protective factor for neurological manifestations.¹⁵ Indeed, the only Type 3 GD patient presented the L444P allele, along with an unknown allele.

The ERT infusion periodicity was constant during all intervals of the treatment. The mean dose administered was slightly higher than the dose recommended by the Brazilian Consensus for the treatment of GD (32.88 IU/kg versus 30.0 IU/kg).⁷ This small difference is due to the fact that the dose registered in patients' records was the total amount and the division in respect to the patients' weight frequently did not result in an exact value. Furthermore, individual variations observed in Table 1 reflect: 1 – the levels of the severity of the disease that require higher or lower doses, as explained by Goker-Alpan;¹⁶ 2 – the need of new adjustments to the dose due to a dramatic reduction in the stock of imiglucerase after a viral infection that occurred in 2009;¹⁷ 3 – the retake of the doses recommended by the Therapeutic Guidelines for GD of the Brazilian Ministry of Health,¹⁸ after the stock was replenished.

In the study sample, ERT was effective in raising the hemoglobin levels in the first two years of treatment and in keeping them stable during all the period that the enzyme was administered. This was also true even when the patients were under 12 years old at diagnosis. The improvement in anemia and the stabilization of hemoglobin levels have already been demonstrated in a two-year study of 148 patients in which the number of anemic patients drastically fell within the first six months of starting ERT.³ The results of the hemoglobin levels at the end of two years of treatment are in accordance with the therapeutic goal proposed by Weinreb et al.,¹⁹ i.e., hemoglobin ≥ 11 g/dL in under 12-year-old patients (87.5% in the current study), ≥ 11 g/dL in adult women (not achieved by only one woman in this study) and ≥ 12 g/dL in adult men (achieved by both men in the current study).

The platelet count also rose significantly by the end of two years of ERT. Weinreb et al.¹⁹ divided the therapeutic goal for platelet count into three groups: patients with count at diagnosis $>120.0 \times 10^9/L$ must remain in this range, patients with counts ranging from $60.0 \times 10^9/L$ to $120.0 \times 10^9/L$ must achieve counts over $120.0 \times 10^9/L$, and patients with counts $<60.0 \times 10^9/L$ must increase their count by twofold. In the study sample, the results were satisfactory, as nine of the 13 patients (69.23%) achieved the therapeutic goal within two years of treatment. For the first two groups, only Patient 1, who belonged to the second group, did not reach the therapeutic goal. However, none of the patients in the third group tripled their platelet counts within two years of ERT. These patients

present a more severe disease and respond slowly and gradually (or even unsatisfactorily) to therapy as far as the platelet count is concerned.⁵

Nonetheless, ERT is capable of keeping the disease stable over the years in most patients. This is evidenced by the normal mean hemoglobin levels and platelet counts at the end of five and ten years of treatment. This effect was observed in a study with 887 children whose hemoglobin levels and platelet counts improved in the first two years and remained stable over eight years of observation.¹⁴ Despite the presence of adult patients in the study sample, our results suggest that the efficacy of ERT is similar in different age groups in relation to these two hematological parameters.

Additionally, the results suggest that a lower-dose regimen (i.e., approximately 30 IU/kg every two weeks) is as effective in reaching the therapeutic goals as a high-dose regimen, as originally proposed by Barton et al.⁸ (60 IU/kg every two weeks). This brings an economic advantage in non-wealthy nations, such as Brazil, allowing them to reserve high-dose regimens for critically symptomatic patients who need a faster recovery, similar to what is reported in Israel.²⁰

The absence of statistical significance in the white cell count is probably due to extremely variable results between individuals, i.e., while some presented leukopenia, others presented leukocytosis. Leukocytosis is not unexpected in GD patients as they are more likely to acquire infections. Furthermore, the leukocyte function is disturbed in GD due to substrate accumulation in these cells, especially the macrophage/monocyte lineage, inherent to the disease physiopathology.²¹ Additionally, leukopenia is usually not severe and rarely requires any intervention, making the white cell count a non-specific parameter in the follow-up of patients.⁶

Similar to the study by Ferreira et al.,¹ splenomegaly was found in 100% of patients (splenectomized patients were not included) when clinical and radiological methods to detect this alteration are considered. Despite the significant reduction in the number of patients presenting with splenomegaly after two years of ERT, it was not possible to determine whether there was a reduction in volume in patients who still presented this alteration, as most records did not state the degree of splenomegaly and, when they did, the data were not standardized, precluding a more accurate analysis. Considering that in subsequent periods there was reduction in the number of patients with splenomegaly, even though not statistically significant, the authors believe a reduction in the spleen volume also occurred in the first two years of ERT, similar to what is reported in the literature.

The number of patients with hepatomegaly had decreased by the end of two years of ERT similar to the study of Sobreira & Bruniera;³ however, in that study, the reduction in liver volume occurred primarily between the 12th and 18th months of ERT. There was no statistically significant reduction in the following periods which shows the early response to ERT in respect to hepatomegaly (within 2 years).

ERT was inefficient to control neurological symptoms in the only Type 3 patient in the study. Symptoms of this patient persisted despite increases in dose. This case exemplifies the higher severity of Type 3 GD as already reported by Goker-Alpan¹⁶ and Cox.¹⁷

Conclusion

ERT has an important impact on improving hemoglobin levels and platelet counts. These parameters respond to treatment in the initial phase of drug treatment (within 2 years) and the response is maintained as long as ERT is administered every two weeks accordingly to the standard regimen proposed in the literature. Hepatomegaly and splenomegaly also respond significantly to ERT within two years of treatment. As splenomegaly is the most frequent manifestation, the presence of this sign in a patient with cytopenia should lead the attending physician to consider GD as one of the differential diagnosis. Due to the wide range of manifestations, the treatment of GD and follow-up must be performed by a multidisciplinary team.

Conflicts of interest

The authors declare no conflicts of interest.

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