Research article

A reappraisal of Gaucher disease patients - Clinical presentation, and diagnosis in rare disease unit of central child teaching hospital in Baghdad province

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ABSTRACT

Introduction and Aim: Gaucher disease (GD) is an autosomal recessive ailment caused due to mutations in the GBA1 gene, encoding for the lysosomal enzyme, glucocerebrosidase. The aim was to evaluate the clinical, biochemical, and molecular parameters associated with this disease, as well as to identify symptoms and covariables thought to be most diagnostic of early GD presentation, allowing for early diagnosis and management.

Methodology: This cross-sectional study involved twenty-six patients diagnosed with GD at the Metabolic Department of Central Child Teaching Hospital, Baghdad, Iraq. Diagnosis depended mainly on history and physical examination and confirmed by beta-glucosidase enzyme assay of dry blood spot on filter paper and Lyso-GL-1 level. Amplification-based next generation sequencing approach was used in investigating the GBA1 gene at the molecular level.

Results: The mean age of the 26 (17 male and 9 female) patients was 9.13 years, with 100% consanguinity and 50% positive family history. The average number of years from the start of clinical manifestations to the diagnosis' validation was 3.82 years. The original age of presentation was 2.83 years, and the initial age of diagnosis was 6.65 years. Hepatosplenomegaly (85%), pallor (88%), splenomegaly (12%), splenectomy (12%), hemorrhage (19%), bone discomfort (23%), bone breakage (12%), and GD type III (19%) were observed. Among patients 65% exhibited radiological bone abnormalities, 54% had Erlenmeyer flask deformity, and 1.5% had osteopenia and fracture. Studies of GBA1 gene showed the mutation C.[1448T>c](P.[Leu483Pro] to be the most common. Hepatosplenomegaly and hematological deficiencies were effectively improved by enzyme replacement therapy.

Conclusion: In Gaucher disease, GBA1 gene mutation analysis could provide some predictive information about disease variance as well as severity. PCV%, platelet count, ferritin, and lyso-GL-1 levels could be employed as biomarkers for the diagnosis of GD. ERT proved effective in treating hematological and hepatosplenomegaly abnormalities.

Keywords: Gaucher disease (GD); Enzyme replacement therapy (ERT); Lysosomal storage disorders (LSDs); glucosidase beta acid (GBA1) gene.

INTRODUCTION

Gaucher disease (GD) is a lysosomal disorder characterized by the accumulation of glucocerebroside in the lysosomal tissue of macrophages due to the absence of the β-glucocerebrosidase enzyme (1). Systemic accumulation of glucocerebroside results in splenomegaly, abdominal discomfort, anemia, thrombocytopenia and coagulopathy or hepatomegaly, and adverse pattern of bone disease (2,3). Autosomal recessive mutations in the GBA1 (glucosidase beta acid 1) gene, located on chromosome 1q21 is responsible for the deficiency of β-glucocerebrosidase enzyme (4). Around 300 mutations and polymorphisms are reported for the GBA1 gene (5). GD is an inherited disorder which is further categorized as Type 1(non-neuronopathic), type 2 (an acute neuropathic form), type 3 (chronic neuropathic) (5,6). In the general population, the prevalence of this disease ranges from 1:40000 to 1:60000 births; however, births in the Ashkenazi Jewish population exceed 1:800 (7,8). Measurement of β-glucocerebrosidase enzyme in blood leukocytes is considered a gold standard for diagnosis of GD (9). At the molecular level, testing for GBA1 gene mutation biomarkers such as chitotriosidase, CCL18, glucosylsphingosine, and ferritin are also used in diagnosis of this disease (10, 11). The enzyme replacement therapy (ERT) using macrophage targeted mannose terminated glucocerebrosidase enzyme is considered the standard of treatment for patients with GD (12). Therapy using oral substrate reduction was found to make significant enhancement in the spleen, size of liver, and hematological factors and other between patients with GD type (13). In this study, clinical, biochemical, and molecular parameters, and management outcome of 26 patients with Gaucher disease were studied and investigated to detect signs and co-variables considered most symptomatic of early presentation of GD, to help non-specialists recognize 'at-risk' patients that may benefit from diagnostic testing.

MATERIALS AND METHODS

This cross-sectional study included 26 GD patients diagnosed at the Metabolic Department of Central Child Teaching Hospital, Baghdad, Iraq. The research was carried out over a seven-year period (from April 2014 to April 2021). For the diagnosis of GD, dry blood spotted on filter paper was sent to Centogene lab Germany, Archimed life lab, Vienna. GD was confirmed based on β-Glucosidase enzyme assay using fluorimetry, and lyso-GL-1 level by using liquid chromatography coupled with mass spectrometry. Mutational analysis of the GBA1 gene was investigated by amplification based next generation sequencing (NGS). All patients signed a written consent prior to participation in the study.

Patient data was collected and included both demographic (age, age of presentation, diagnosis and starting treatment, gender, consanguinity, and family history) and clinical data (History and physical examination of liver and spleen before and during treatment, growth, development and neurological assessment). Every six months, blood was collected from each patient and tested for PCV%, platelet count, ferritin level, and Lyso-GL-1. Each participant was also subjected to abdominal ultrasound and bone X-ray every 6 months.

Statistical analysis

The data obtained is expressed as means ± SD. Statistical analysis was carried out using the SPSS ver. 24 software. Qualitative features such as clinical and skeletal results are presented as frequency distribution data.

RESULTS

The study comprised 26 Gaucher disease patients (17 men and 9 women) ranging in age from 2.5 to 33 years. The consanguinity among parents was 100% with 50% having a family history of the disease (Table 1). The mean age for initial clinical manifestations of the disease among the 26 patients was 2.83 years (SD 3.42; rang 0.25-12 years) with 8 patients being < 1year, 17 patients from 1-10 years, 1 patient > 10 years. The average age of the patients was 6.65 years (SD 8.38; rang 0.25-33 years), 2 patients <1 year, 13 patients between 1-5 years, 9 patients from >5-15 years and 2 patients > 15 years, as shown in Fig. 1.

Table 1: Demographic data of GD patients in the study

Age of patients in years						**Positive family history**	**Consanguinity**
1-10 years		**<10-20 years**		**> 20 years**		13/26 (50%)	26/26 (100%)
M	F	M	F	M	F		
12/26 (46%)	8/26 (31%)	3/26 (12%)	1/26 (4%)	2/26 (8%)	0/26		
Total no. 20/26 (77%)		Total no.4/26 (15%)		Total no.2/26 (8%)			

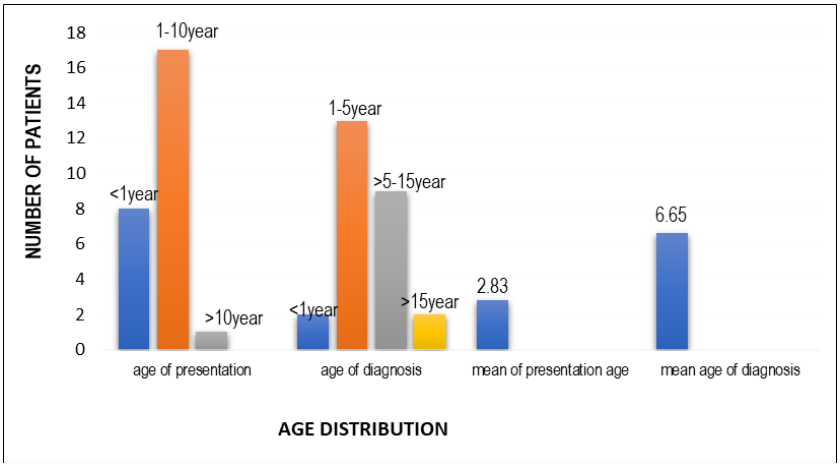

Fig. 1: Age distribution of studied patients based on presentation and age of diagnosis

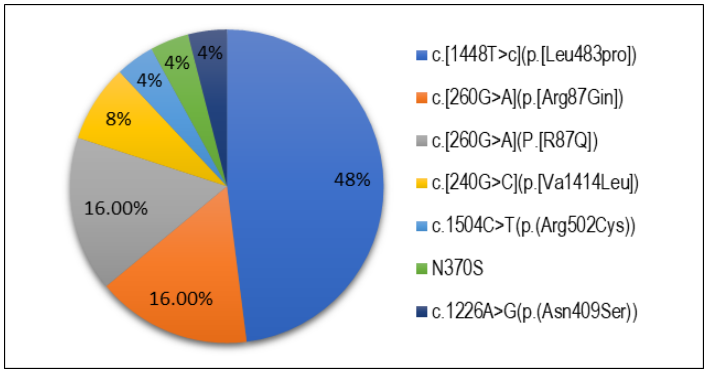

Fig. 2: Types of molecular mutations within the GBA1 gene

The GBA1 mutational analysis was undertaken for 25 out of the 26 patients with GD the most prevalent mutation among patients was c.[1448T>C] (p. Leu 483pro] (48%), followed by c.[260G>A](p.Arg87Gin) (16%), c.[253G>A] (P.Gly85Arg) (16%), c.[240G >C C] (p.Va1414Leu) (8%), c.[1504C>T](p.(Arg502Cys) (4%), N370S (4%), and c.[1226A>G] (p. Asn409Ser) (4%) (Fig. 2).

Hepatosplenomegaly as an initial presentation was observed in 22 out of 26 patients (85%), while 3 patients had history of splenectomy before confirmation of diagnosis (12%), 3 patients (12%), had just splenomegaly at time of presentation and one of our studied patients was a new born brother of an affected patient. Hepatosplenomegaly was accompanied by pallor in 23 patients (88%), and bleeding in 5 patients (19%), mainly as epistaxis. Skeletal involvement, in addition to hepato-splenomegaly and pallor, was observed in 9 patients, as follows: 6 patients (23%), had a history of severe bone pain, and 3 patients (12%) had a history of bone fracture, primarily of the femoral bone. Five patients (19%) were diagnosed as having type III Gaucher disease, who presented different neurological sign and symptoms in addition to hepatosplenomegaly and pallor as follow, (one had history of epilepsy on anticonvulsant 4%, 4 patients with squint 15%, one patient had tremor 4%, and 3 patients had delay milestones 12%), as shown in Fig. 3.

Five patients (19%) were diagnosed with type III Gaucher disease, with various neurological signs and symptoms in addition to hepatosplenomegaly and pallor (Fig. 3) among which one patient had epilepsy and was on anticonvulsants, four patients with squint, one patient with tremor, and three patients had delayed milestone (Fig. 3).

Association for main sign and symptoms exhibited by patients to the mutation types, showed hepatosplenomegaly and pallor to be chiefly associated with the mutation c.[1448T>c] and to a lesser extent to the mutation [c. 260G>A (Table 2). Other symptoms such as bone pain, bleeding and other bone problems were also seen to be associated with the [c.1448T>c] mutation (Table 2). Splenomegaly was seen to be present only in patients exhibiting the c [260G>A], [C. 253G>A] and c.[1226A>G], mutation type. One patient with a history of bleeding mainly as epistaxis and one with bone pain was associated with the mutation [c.240G>C] and [c.260G>A] respectively (Table 2). Five patients diagnosed as having type III Gaucher disease presented different neurological signs and symptoms, four of them had [C.1448T>c], genotype and another one had N370S type of mutation, as summarized in Table 2.

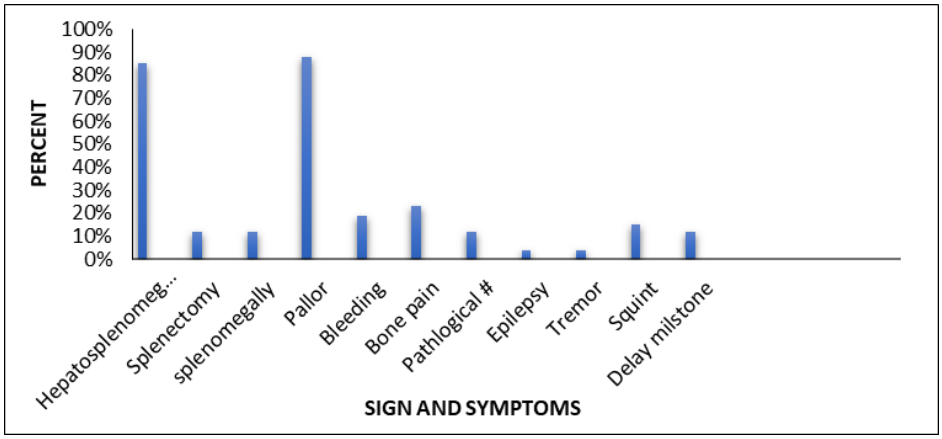

Fig. 3: Clinical presentation of studied patients

Table 2: Distribution of symptoms and signs with regard to mutation type

No. of patients/ 26	Mutation type Amino acid change	HSM+	Splenomegaly	Splenectomy	Pallor	Bleeding	Bone pain	Bone fracture	Neurological signs and symptoms
12/26	c.[1448T>C] p.[Leu483Pro]	12/12(100 %)	-	2/12(16.6%)	12/12(100%)	3/12(25%)	5/12(42%)	2/12(16 %)	4/12(33%)
4/26	c. [(260G>A] p.[Arg87Gin]	4/4(100%)	-	1/4(25%)	4/4(100%)	-	-	1/4(25%)	-
4/26	c.[253G>A] p.[Gly85Arg]	1/4(25%)	3/4 (75%)	-	2/4(50%)	-	1/4(25%)	-	-
2/26	c.[240G>C] p.[Va1414Leu]	2/2(100%)	-	-	2/2 (100%)	2/2(100%)	-	-	-
1/26	c.[1504C>T] p.[Arg502Cys]	1/1(100%)	-	-	-	-	-	-	-
1/26	N370S	1/1(100%)	-	-	1/1(100%)	-	-	-	1/1(100%)
1/26	c.[1226A>G] p.[Asn409Ser]	-	1/1(100%)	-	1/1(100%)	-	-	-	-
1/26	No mutation	1/1(100%)	-	-	1/1(100%)	-	-	-	-

HSM +: hepatosplenomegaly

Table 3: The relation of baseline laboratory finding to the type of mutations

Lab. parameters	Frequency	Mean	SD	Min	Max
PCV%	26	27.3	4.06	22%	36%
c. [1448T>c]	12/26	25.8	2.51	22%	30%
c. 260G>A]	4/26	26.7	5.61	22%	33%
c. [253G>A]	4/26	31	5.29	30%	36%
c. [240G>C]	2/26	26.5	2.12	25%	28%
c.[1504C>T]	1/26	35	35	35%	35%
N370S	1/26	28	28	28%	28%
c.[1226A>G]	1/26	24%	24%	24%	24%
No mutation	1/26	30	30	30%	30%
Platelet x 10^3/L	26	101.1x10^3x/L	49.2x10^3/L	39x10^3/L	262x10^3/L
c. [1448T>c]	12/26	75	22.2	39	111
c.[260G>A]	4/26	90.5	30.5	63	123
c. [253G>A]	4/26	180.75	63.6	122	262
c. [240G>C]	2/26	114	14.1	89	109
c.[1504C>T]	1/26	122	122	122	122
N370S	1/26	153	153	153	153
c.[1226A>G]	1/26	122	122	122	122
No mutation	1/26	51	51	51	51
Ferritin ng/ml	26	618.6	473.9	65.8 ng/ml	1650 ng/ml
c.[1448T>c]	12/26	741.8	514.4	185	1650
c.[260G>A]	4/26	507.4	489.8	156	1232
c. [253G>A]	4/26	377.3	320.8	167	854
c. [240G>C]	2/26	602	584	189	1015
c.[1504C>T]	1/26	1365	1365	1365	1365
N370S	1/26	697	697	697	697
c.[1226A>G]	1/26	65.8	65.8	65.8	65.8
No mutation	1/26	311	311	311	311
Lyso-GL-1	26	700.4	454.4	281.8 ng/ml	1852.9ng/ml
c. [1448T>c]	12/26	741.5	398.9	329	1560
c.[260G>A]	4/26	919.8	505.7	417	1578.2
c. [253G>A]	4/26	369.8	61.3	281.8	421.9
c. [240G>C]	2/26	713.6	2.19	712.1	715.2
c.[1504C>T]	1/26	1852.9	1852.9	1852.9	1852.9
N370S	1/26	324.5	324.5	324.5	324.5
c.[1226A>G]	1/26	550.8	550.8	550.8	550.8
No mutation	1/26	0	0	0	0

Cut-off values for PCV% =34-40%; Platelet =200-490 x10^3/L; Ferritin = 200 500 ng/ml (1 month), 50 200 ng/ml (2 -5 months), 7-140 ng/ml (6 months-15 years) and 15-200 ng/ml (>15 years); Lyso-GL-1 = 0.0-14 ng/ml

The initial mean for PCV%, platelet count, ferritin level and Lyso-GL-1, as a base line before (ERT), for all types of mutation was as follows: 27.3 (SD 4.06; rang 22%-36%), 101.1 x 10^3/L (SD49.2; rang 39-262 x 10^3/L), 618.6 ng/ml (SD 473.9; rang 65.8-1650 ng/ml), and 700.4 ng/ml (SD 454.4; rang 281- 1852.9 ng/ml), respectively (Table 3). Anemia was documented in 23/26 (88%) of patients, with two patients assigned with the mutation C.[253G>A], and one patient with c.[1504C>T]. The genotype having normal PCV%, while thrombocytopenia was detected in all types of mutation except two patients assigned the mutation c. [260G>A]. According to ferritin and Lyso-GL-1 all types of mutations had very high levels as initial base lines, as shown in Table 3**.**

Radiological bone abnormalities were documented in 17/26 (65%) of the patients studied; fourteen patients (54%), from various types of mutation, had Erlenmeyer deformity, while three patients (11.5%) had osteopenia and fracture, one of whom was an adult with c. [260G>A], genotype, and another two patients had c.[1448T>c], genotype. Thirteen patients (50%), from various types of mutations, had a bone marrow biopsy that revealed Gaucher cells. All 26 patients showed reducing in the activity of β-glucocerebrosidase enzyme, (80.6%) with residual enzyme activity (REA) below 50% of cut-off value, five patients(19%) had (REA) above 50% of cut-off value, all of them from c. [1448T>c], type of mutation but had severe and early clinical manifestation, while two patients who was share c[.260G>A] and c.[253G>A] type of mutation had no enzyme activity

but they had mild and late onset of clinical manifestation.

Twenty-six patients were given enzyme replacement therapy (ERT) every other week by intravenous infusion for the rest of their lives at a recommended dose of 60 Units/Kg. The average age of initial ERT was 7.12 years (SD 8.31; range 0.5–33 years). There was a 0.47-year wait between diagnosis and ERT starting, owing primarily to the discontinuity of ERT in our nation. In our hospital, ERT for Gaucher disease began in 2014, and therefore monitoring of ERT response was for the last ten months. The laboratory parameters PCV%, platelet count, ferritin level and Lyso-GL-1 level were monitored every six months during ERT. The mean liver span before ERT, clinically was 8.34 cm under the right costal margin (SD 4.85 cm; range 4–17 cm), by ultrasound. The liver span showed an average of 10.73 cm at the mid clavicular line (SD 6.23; range 4–21 cm) and were all abnormal for sex and age. Clinical average of splenic span before ERT was 12.23 cm (SD 7; range 5–25) below the left costal margin. The ultrasound splenic spread was 12.55 cm (SD 7.17 cm; range 7-27 cm), which was all above the maximum limit for age and gender. After initiating ERT, we discovered that the mean span for the liver clinically and by abdominal ultrasound was 3.88cm (SD3.34; rang 0-10cm) and 4.24cm (SD 4.26; rang 0-12cm), respectively, whereas the splenic span after ERT clinically was 5cm (SD 5.66; rang 0-20cm) and 6cm (SD7.28; rang 0- 21cm), respectively.

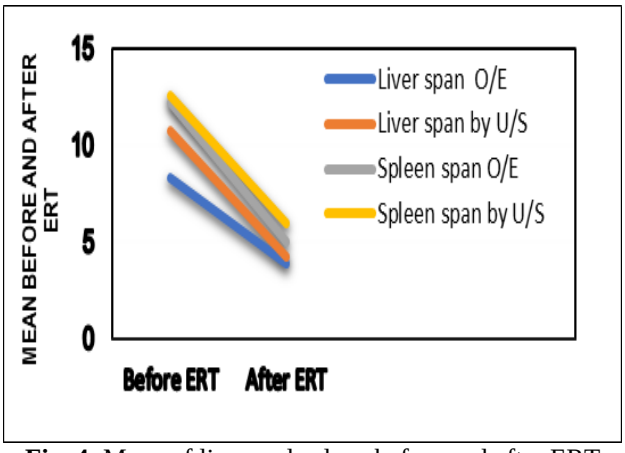

Fig. 4: Mean of liver and spleen before and after ERT

The laboratory parameters before and after ERT were measured. Prior to ERT, the PCV% was 27.3(SD 4.06; rang 22-36%), platelet 101.1 (SD49.2; range 39-262), ferritin was 618.6 ng/ml (SD 473.9; range 65.8-1650 ng/ml), and baseline mean of Lyso-GL-1 was 700.4 ng/ml (SD 454.4: range 281-1852.9 ng/ml). After ERT the mean values obtained was 34.8 (SD 3.91; range 28-43), 198.7(SD 79.7; range 65-381), 99.4(SD 126; range 10-650), 282(SD 280.2; range 69-933) for PCV%, platelet, ferritin, and Lyso-GL-1, respectively (Fig. 5).

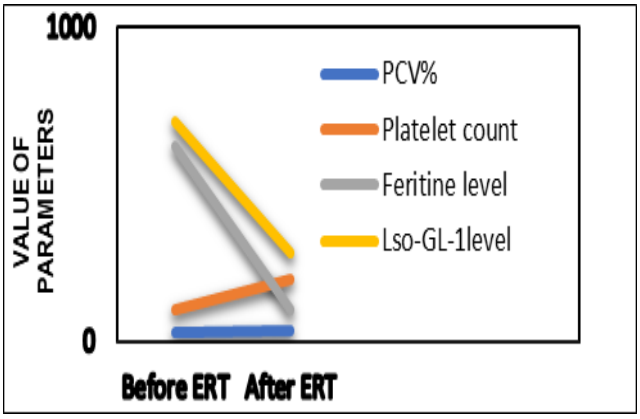

Fig. 5: Value of main parameters before and after ERT

DISCUSSION

More than 45 different lysosomal storage disorders (LSDs) are currently recognized as metabolic illnesses among which the most common is Gaucher disease (14). It is crucial to recognize the early signs of the disease in order to reduce its adverse effects. To raise the level of knowledge among clinicians regarding this disease, whose presentations overlap with those of other more prevalent hematologic and oncological disorders, we reviewed the primary clinical presentation of our Gaucher disease patients in this study.

This study showed that the average age of patients was 2.83 years (SD 3.42; range 0.25-12 years), while the mean age of diagnosis was 6.65 years (SD 8.38; range 0.25-33 years), and there was average of 3.82 years delay from the start of clinical appearances to the verification of diagnosis for patients which could probably be attributed to the delay in diagnosis. However, our findings are consistent with those of a previous study (15), which found that the majority of patients with type 1 disease have an onset in the first decade of life, i.e. 1.12 years (SD 0.56: 0.5-2 years), and the mean onset age for type 3 patients was 1.7 years (SD 1.19: 1-4), with a mean of 2.2 years from the beginning of clinical manifestations to confirmation of diagnosis for all types. All patients had a high consanguinity rate and a positive family history, which agrees with (16,17), but disagrees with (15), who had all their patients from non-consanguineous pedigrees.

In this study, the frequency of c[.1448T>C] (amino acid change being Leu483Pro) mutation within the GBA1 gene was the highest, accounting to 48%. This is in agreement with previous studies, wherein this mutation was reported to be the most common (15, 18). The frequency of specific mutations has been shown to vary between populations. For example, in Ashkenazi Jewish ancestry, the mutation, N370S (c.1226 A>G) was shown to be most prevalent (19), while in this study only one patient showed this mutation.

The current investigation revealed that hepato-splenomegaly linked to hematologic, bone, and

neurological symptoms, to be the most frequent clinical phenotype and agrees with earlier studies (15,18).

Hepatosplenomegaly was accompanied by pallor (88%), and bleeding (19%) in this study, which also agrees with similar studies undertaken on the Filipino population (15). Type III Gaucher disease was diagnosed in 19% of patients in this study, who in addition to hepatosplenomegaly and pallor showed symptoms of epilepsy, squint, tremor, and delay milestone. Stirnemann *et al* (18) in their study revealed GD patients in addition to HSM, to exhibit symptoms of epilepsy, strabismus, and intellectual disability. As demonstrated by Alfonso *et al.,* (19) the expression of the mutant allele on phenotype in various populations could probably be due to ethnic diversity and some effect of the modifier gene on the mutant allele. One of the patients had a history of splenectomy and bone fracture, which shows GD patients are at a risk for developing bone disease as demonstrated earlier (20). Our findings are also consistent with earlier research indicating that GBA1 gene mutation analysis in case of GD could provide some predictive information about the variation as well as the severity of disease (18, 21,22).

Biochemical abnormalities are common in Gaucher disease (20). Biochemical diagnosis of GD in this study, ascertained elevated levels of PCV%, platelet count, ferritin, and lyso-GL-1 levels before ERT, which is consistent with earlier research that found these biochemical abnormalities to be frequently associated with Gaucher disease and could thus be used as a specific biomarker for diagnosis and monitoring of all GD patients (18,19,23). Further, the 26 patients who underwent compassionate ERT were monitored for these biochemical parameters every six months till the end of ERT. We observed that patients who began ERT early to have normalized liver and splenic spans, whereas patients who began ERT in late still continued having HSM, even though their liver and splenic spans had decreased but did not reach the normal limit for age and gender which is in line with previous studies (15,18). This indicates that use of ERT can change the clinical phenotype of GD, lowering the severity of the disease. However, the neurologic symptoms of the patients studied were not relieved by ERT, probably because the enzyme did not cross the blood brain barrier, as reported in prior studies (15,18). Although this study has limitations due to the small population size, it appears that physical, biochemical, and molecular parameters could be used to detect early signs of GD and 'at-risk' patients, thus facilitating appropriate treatment.

CONCLUSION

This study found that all of the individuals evaluated had type 1 diabetes, which was verified by the prevalence of severe mutations and clinical presentation observed in the studied group. GBA1 gene mutation analysis in case of GD could provide some predictive information about the variation as well as the severity of disease. Biochemical parameters such as PCV%, platelet count, ferritin and lyso-GL-1 levels could be used as biomarkers for diagnosis of GD. ERT successfully improved hematological and hepatosplenomegaly abnormalities.

CONFLICTS OF INTEREST

Authors declare that there is no conflict of interest.

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