

## Twenty-Five Years of Biochemical Diagnosis of Gaucher Disease: The Egyptian Experience

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### Abstract

**Background:** Gaucher disease is a rare multi-systemic metabolic disorder resulting from the deficiency in  $\beta$ -glucocerebrosidase enzyme, with consequent accumulation of glucocerebroside. Less than 15% of mean normal activity  $\beta$ -glucocerebrosidase in leukocytes is the gold standard for the diagnosis of Gaucher disease, which is supplemented by a massive elevation in chitotriosidase enzyme activity. We report here our experience in the biochemical diagnosis of Gaucher disease by showing the variability and the heterogeneity of the activity of enzymes over 25 years from 1993-2017, through referring 5128 clinically suspected Gaucher disease cases to our Biochemical Genetics Department, National Research Centre, as the main reference lab in Egypt for the diagnosis of Inherited Metabolic Disorders.

**Methods:**  $\beta$ -glucosidase enzyme activity and chitotriosidase were done to all referred cases. Sphingomyelinase activity was estimated for all cases with normal activity of  $\beta$ -glucosidase and moderate elevation of chitotriosidase.

**Results:** Out of the 5128 suspected cases, 881 (17%) had deficiency in  $\beta$ -glucocerebrosidase activity, accompanied with high chitotriosidase activity level, ranges (213-66700  $\mu\text{mol/l/h}$ ) and mean (7254.8  $\mu\text{mol/l/h}$ ). Zero chitotriosidase activity is found in 9 patients (1%) with low  $\beta$ -glucosidase. 451 cases were diagnosed as Niemann Pick patients (8.8%).

**Discussion and Conclusion:** Other biochemical markers are needed in addition to chitotriosidase enzyme for the diagnosis. Molecular testing was done in relatively small numbers and need to be available parallel with biochemical testing.

**Keywords:** Gaucher Disease;  $\beta$ -glucocerebrosidase; Chitotriosidase; Egyptian experience

### Introduction

Gaucher disease (GD; MIM #230800) is the most common lysosomal storage disorders. Primarily resulting from a deficiency in glucocerebrosidase enzyme activity (acid beta glucosidase; *GBA*; EC 3.2.1.45) which, leads to accumulation of undegraded glucocerebroside in several tissues, mainly in mononuclear origin of cells or secondarily due to mutations in the sphingolipid activator protein *SAP C* gene [1,2]. The disease prevalence in general population is between 1:20,000 and 1:200,000, with a high prevalence in the Ashkenazi Jewish population. (1:450) [3].

GD presents as three variants according to the clinical symptoms and the onset of disease: GD type I Non-neuronopathic (OMIM# 230800), GD type II acute neuronopathic, and GD type III subacute neuronopathic GD type III is mainly seen in Northern Europe, Egypt and East Asia [4-9]. The location of glucocerebrosidase gene (*GBA*) is on chromosome 1q21 [10]. The most common four mutations are N370S, IVS2 (+1), 84GG and L444P.

The standard biochemical diagnosis of GD is by demonstrating beta-glucocerebrosidase enzyme deficiency in peripheral blood leukocytes using the substrate 4-methylumbelliferyl- $\beta$ -D-glucopyranoside, or through enzymatic analysis of fibroblasts cultured from skin biopsy specimens. And recently also, in dried blood spot using synthetic substrates and tandem mass spectrometry [11,12]. In affected cases the activity of acid glucosidase is about 0%-15% of normal activity; however enzyme activity cannot detect the disease phenotype or heterozygote carriers of GD nor of saposin C deficiency [11].

Increased chitotriosidase activity is an epiphenomenon resulting from activation of macrophages upon uptake of glucosylceramide, so

it is abundantly expressed in lipid macrophages and used as biomarker for GD since 1994 by Dr. Hollak and co-workers and also used to reflect the total storage of Gaucher cells and response to therapy [13].

Although GD patients display a massive elevation in chitotriosidase, reach to 10-100-fold, other lysosomal storage diseases may show elevated levels to a lesser extent [14]. Furthermore, some individuals including GD patients (4-5%) are homozygous for 24 bp duplication in exon 10 chitotriosidase gene which renders the enzyme inactive. So, another biochemical marker will have to be tested [15]. Pulmonary and activation-regulated chemokine (CCL18/ PARC) are elevated to 10-40-fold in symptomatic GD patients and its urine level is well related to GD patients than chitotriosidase level [16-18].

Gaucher cells, such as activated macrophages, are responsible for several cellular responses manifested in GD, like cytokines (interleukin-1b, interleukin-1 receptor antagonist, interleukin-2 receptor, CD14 and M-CSF) [19,20]. In 2013, Rolfs et al. have proposed that, glucosylsphingosine is a more specific and sensitive biomarker than CT and CCL18/PARC in normal individuals, GD cases, GD carrier and other LSD patients [21]. Alternative ancillary biomarkers

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comprise increased activities/concentrations of tartrate-resistant acid phosphatase, angiotensin converting enzyme (ACE), and plasma ferritin [22]. Confirmation and better understanding of cases can be afforded through molecular analysis of human *GBA* gene [23]. The prenatal diagnosis is based on enzymatic analysis using fetal cells from chorionic villus at 10-11 weeks of pregnancy. Knowledge of the proband's mutation or heterozygous parent's mutation would also allow the use of DNA mutation for prenatal analysis [24].

The aim of the treatment is to reduce the excessive amount of glucocerebroside and other glycolipids. There are four routes for treatment: Enzyme replacement therapy, substrate synthesis inhibition therapy (SRT), pharmacological chaperone (PC) therapy, and gene therapy [25-30]. The aim of this study focuses on five different points one to describe our biochemical experience and recommendations in the diagnosis of Gaucher disease through 1993-2017 as a guide for an effective diagnosis, second to enrol all previously diagnosed cases and their total number over 25 years from 1993 to 2017, third to give an overview of the annually diagnosed cases, fourth to estimate approximately the incidence of the diagnosed cases among our population and fifth to compare the number of diagnosed cases versus the cases under treatment.

## Materials and Methods

From March 1993 till December 2017, the Biochemical Genetics lab. the reference laboratory for inborn errors of metabolism for Egypt at National Research Centre, received blood samples from 5128 patients with suspected Gaucher signs and symptoms. These samples came from various areas throughout Egypt.

## Preparation of the samples

5 ml of whole blood samples were collected by venous puncture in EDTA tube. Plasma was obtained for CT assay and leukocytes were separated to determine the activity of  $\beta$ -glucosidase implies you are using the synthetic substrate. Leukocytes were isolated from peripheral blood according to the method described by Skoog and Beck [31]. Plasma was obtained through centrifugation (2,500 rpm for 5 min). Before initiating enzymatic analysis, proteins content was estimated according to Lowry et al. [32]. For all the cases the measurement of  $\beta$ -glucosidase activity was done with the assessment of chitotriosidase and finally sphingomylinase activity in peripheral leucocytes. The normal ranges for chitotriosidase is 4-80  $\mu\text{mol/l/h}$ , for  $\beta$ -glucosidase is 1-5  $\mu\text{mol/g prot/h}$  and for sphingomylinase is (9- 47  $\mu\text{mol/g prot/h}$ ). All the chemicals were purchased from Sigma- Aldrich, St. Louis, MO, United States.

Assay of  $\beta$ -glucosidase activity was with fluorogenic substrate 4-methyl-umbelliferyl- $\beta$ -D-glucopyranoside in the presence of pure sodium taurocholate (2.5 mg/ml) and Triton X-100 (2 mg/ml), the pH was 5.4 using citrate-phosphate buffers. Fluorescence interpretation was performed using a spectrofluorometer at a wavelength of 450 nm of emission and 365 nm of excitation. Activity was expressed in  $\mu\text{mol/g prot/h}$  [33].

Chitotriosidase activity was measured by incubating plasma with 4-methylumbelliferyl- $\beta$ -D-N,N,N'-triaacetylchitotriose (4 MU-chitotriose) as substrate in citrate/phosphate buffer pH 5.2, at 37°C. In GD patients, samples were diluted in demineralized water before incubation. After 15 min the reaction was stopped with carbonate buffer, pH 10.6. Fluorescence reading was performed using a spectrofluorometer at a wavelength of 450 nm of emission and 365 nm of excitation. The result was expressed in  $\mu\text{mol/l/hr}$  [13].

## Results

The present study included a total number of 5128 patients suspected to have Gaucher disease referred to the Biochemical Genetics Department from 1993 to 2017. Age range was (3 months to adulthood), the positive parental consanguinity was present in 3897 cases (76%) of the studied group (Table 1).  $\beta$ -glucocerebroside and Chitotriosidase activity were determined for all suspected cases to diagnose Gaucher patients. Sphingomylinase activity was determined to all non-Gaucher patients, with elevated Chitotriosidase activity.

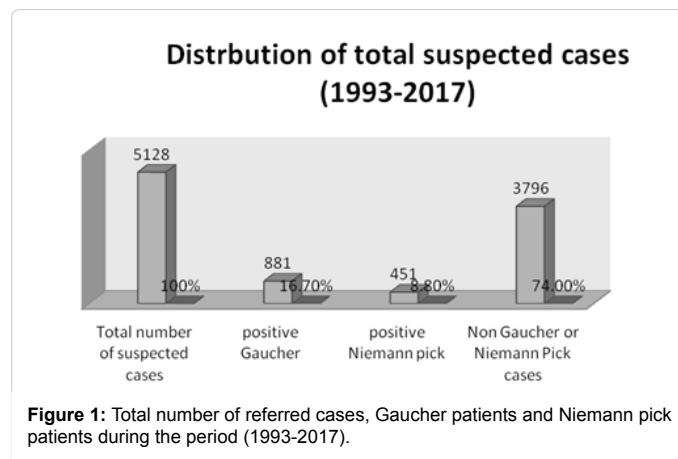
Out of 5128 suspected cases, 881 were diagnosed as Gaucher patients (17%), 719 showed positive parental consanguinity (81.6%), with male to female ratio (1.6:1) in Table 1, Figures 1 and 2 respectively. The age range of diagnosed Gaucher patients was (3 months - 45 years), the age of majority of patients (859, 97.5%) ranged (1.7: 8 years), followed by 16 patients (1.8%) with a range (25: 45 years) and six patients (0.7%) aged below 2 years (3 months: 1.4 years) (Table 2). 451 cases out of 5128 suspected cases were diagnosed as Niemann Pick patients (8.8%) (Figure 1). large number of suspected cases was referred in the years 2014, 2015, 2016 and 2017 while the percent of diagnosed cases was not equally high. In the years 1999, 2000 and 2001 the number of referred cases was not that high but on the other hand the number of diagnosed cases was high (Tables 3 and 4) (Figure 3).

Although most of the patients (766, 87%) revealed a decrease in  $\beta$ -glucocerebroside activity with a mean of (0.3  $\mu\text{mol/g prot/h}$ , 30% of low normal value), still some cases (107, 12.1%) showed a decrease to 66% of low normal value (0.66  $\mu\text{mol/g prot/h}$ ) accompanied with high mean chitotriosidase level (9655  $\mu\text{mol/l/h}$ ) and normal level of sphingomylinase (Table 5). The level of chitotriosidase was dramatically increased in all diagnosed cases with range (213-66700  $\mu\text{mol/l/h}$ ) and mean value (7254.8  $\mu\text{mol/l/h}$ ,  $\pm$  20211.38  $\mu\text{mol/l/h}$ ).

A group of suspected cases (103.2%) showed an elevation in chitotriosidase level with mean (131.8  $\mu\text{mol/l/h}$ ,  $\pm$ 24  $\mu\text{mol/l/h}$ ), normal activities of  $\beta$ -glucocerebroside with mean (3.2  $\mu\text{mol/gprot/h}$ ) and normal sphingomylinase activity. Out of 881 cases 9 patients (1%)

Variables	Number of consanguineous cases	The percent of consanguinity
Out of 5128 suspected cases	3897	76%
Out of 881 diagnosed cases	719	81.6%

**Table 1:** The consanguinity among suspected and diagnosed Gaucher disease patients.



**Figure 1:** Total number of referred cases, Gaucher patients and Niemann pick patients during the period (1993-2017).

showed a zero chitotriosidase activity, accompanied with low activity of  $\beta$ -glucocerebrosidase enzyme as well (Table 5). Figures 4 and 5 showed reduction in chitotriosidase activity in 28 patients under treatment. The range of chitotriosidase activity at time of diagnosis was (367-29636  $\mu\text{mol/l/h}$ ), with mean (5185  $\mu\text{mol/l/h}$ ,  $\pm 7461.4 \mu\text{mol/l/h}$ ) while after the last time for follow-up the range was (38-3572  $\mu\text{mol/l/h}$ ), and mean was 904  $\mu\text{mol/l/h}$ ,  $\pm 1112.3 \mu\text{mol/l/h}$ ).

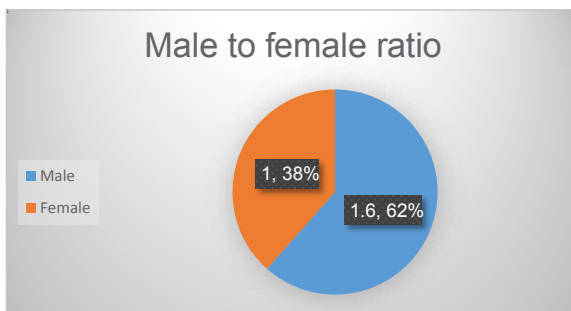


Figure 2: Male to female ratio in diagnosed Gaucher patients during the period of the study (1993-2017).

Age range	Number of patients	Percent (Out of 881 cases)
(3 months: 1.4 years)	6	0.7%
(1.7: 8 years)	859	97.5%
(25:45 years)	16	1.8%

Table 2: Age ranges of diagnosed Gaucher patients through the years of study (1993-2017).

Years	Referred cases	Diagnosed GD cases	Percent of diagnosed cases
1999	37	13	35%
2000	100	25	25%
2001	179	43	24%
2014	424	59	14%
2015	430	61	14%
2016	551	68	12.3%
2017	710	136	19.2%

Table 3: The number of referred and diagnosed cases with the percent of diagnosed cases in certain year.

Table 4: Number of referred cases diagnosed GD patients and their percent during the years of study (1993-2017).

Years of study	Number of Referred cases	Number of Diagnosed GD patients	Percent % of positive GD cases/year
1993	15	3	20
1994	20	3	15
1995	25	4	16
1996	30	5	16.6
1997	36	5	13.8
1998	40	7	17.5
1999	37	13	35
2000	100	25	25
2001	179	43	24
2002	146	31	21.2
2003	257	49	19.1
2004	172	27	15.7
2005	146	33	22.6
2006	165	32	19.4
2007	150	27	18
2008	212	35	16.5
2009	203	51	25
2010	116	17	14.7
2011	237	46	19.4
2012	347	45	13
2013	380	56	14.7
2014	424	59	14
2015	430	61	14
2016	551	68	12.3
2017	710	136	19.2

Number of cases	Normal level of $\beta$ -glucocerebrosidase (1-5 $\mu\text{mol/g prot./h}$ ) (Mean)	Normal level of chitotriosidase (4-80 $\mu\text{mol/l/h}$ ) (Mean)	The Percent out of 881 cases
9	0.45	Zero	1%
766	0.3	6243	87%
107	0.66	9655	12.1%

Table 5: Level of glucocerebrosidase and chitotriosidase enzymes activity in diagnosed Gaucher patients.

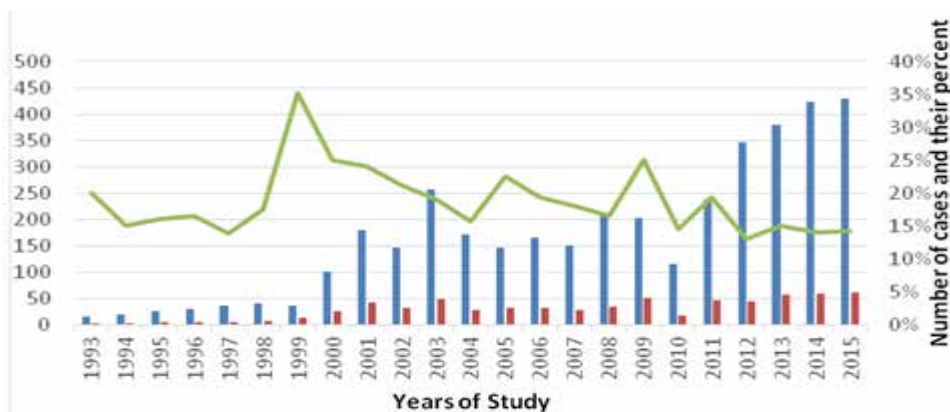
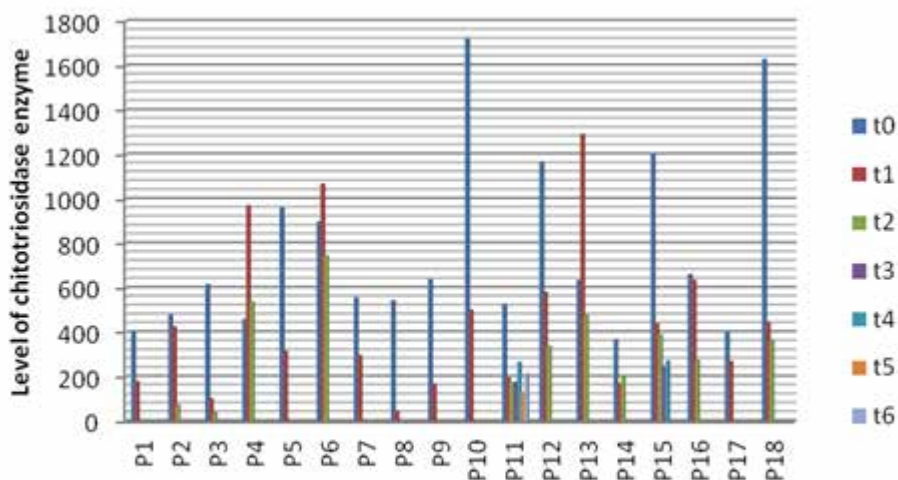


Figure 3: Number of diagnosed cases and their percent in years of study.



Note: t0: Time at diagnosis, t<sub>1</sub> to t<sub>6</sub>: Times of follow-up at different intervals.

Figure 4: A reduction in chitotriosidase enzyme activity in some cases under treatment.

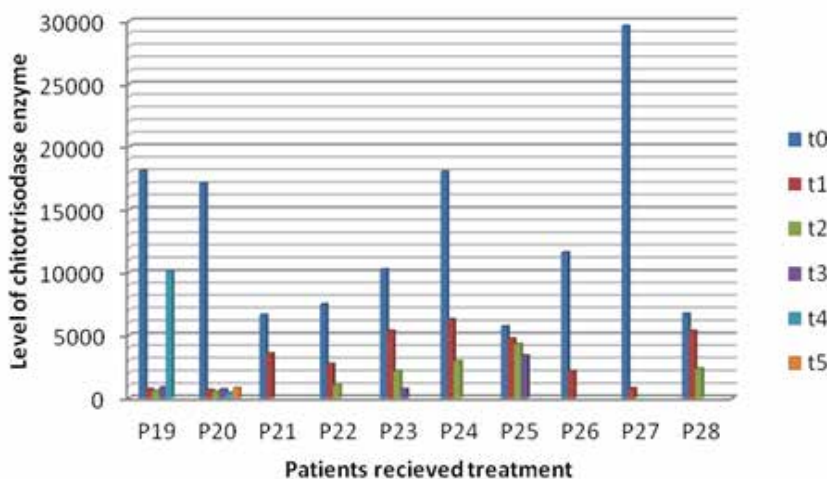


Figure 5: A reduction in chitotriosidase enzyme activity in some cases under treatment.

## Discussion

Gaucher disease (GD) is a chronic condition caused by a recessively inherited deficiency of the lysosomal enzyme  $\beta$ -glucocerebrosidase (glucosylceramidase; EC 3.2.1.45) [1]. The disease is characterized by the accumulation of storage materials, predominantly glucosylceramide, in the lysosomes of the cells of the monocyte-macrophage system. It can be categorized into three types based primarily on phenotypic difference. The most common form seen in Europe and North America is type GD I, which accounts for ~95% of all cases and is conventionally characterised by absence of early onset and primary central nervous system disease [3,4].

Types II and III GD are neuronopathic forms of the disease in which the central nervous system is affected [1]. Type II (acute neuronopathic) GD, the rarest form, affecting <1% of all GD patients, is characterised by rapid deterioration, with death usually occurring before 2 years of age, while patients with type III (chronic neuronopathic) GD (~5% of all GD patients) experience a slower disease course [5].

GD affects all ethnic groups, but it is most prevalent in Ashkenazi Jewish ancestry with a prevalence of 1 in 450 compared with <1 in 100,000 in non-Jewish populations [2,3]. The laboratory diagnosis of GD is based on the measurement of  $\beta$ -glucosidase activity (1) and is assisted by a biochemical marker, the measurement of the enzyme chitotriosidase activity (CT) [13]. In the 90<sup>th</sup> decade, different investigations showed that GD patients had elevated levels of plasma CT (approximately 100–500 times) and that these levels lowered considerably after the introduction of enzyme replacement therapy. Indeed, several indicators of macrophage activation, including CCL18, angiotensin-converting enzyme and cathepsin S, have been identified in excess in the plasma of patients with GD [1]. Confirmation and better characterization of the condition may subsequently be afforded by molecular analysis of the human *GBA* gene. This study illustrates the biochemical experiences in the diagnosis of Gaucher disease at Biochemical Genetics Department, National Research Centre, the main reference lab in Egypt for the diagnosis of inherited metabolic disorders. All cases had been referred from different hospitals and

diagnostic centers throughout Egypt. The age range in this study is 3 months to adulthood. Most of the patients, 859 (97.5%) referred to the lab. were around the age of 2 years with early onset of mild neurological signs.

Many adult cases with GD have been reported worldwide. About 14% of GD patients are diagnosed between the age of 31 and 50 years [34]. In this study, 16 patients only (1.8%) were diagnosed between the ages 25-45 years, with mild type of GD pointing to type I. For GD type II, which is the rarest form, death usually occurs before 2 years of age, 6 cases (0.7%) were diagnosed as GD type II in this study [35].

The fact that most of the diagnosed patients (97.5%) were around the age of two years reflects severe disease with performed anemia, thrombocytopenia, organomegaly and sometimes neurologic manifestations. 1.8% adult patients on the other does not reflect the real number of adult Gaucher patients in Egypt, it only reflects that adult GD are under diagnosed either because of the mild course of the disease or low awareness by the physician in a country like Egypt with so many other causes of hepatosplenomegaly. But from our experience it is mostly due to the mild form of disease in the adult diagnosed cases. 0.6% of the diagnosed Gaucher patients were type II, also does not reflect the real number of type II patients, who die very early due to severe neurologic manifestations without being diagnosed.

These two types (the adult GD form and the acute neuronopathic form) need more awareness among the physicians to estimate the real number of these patients among our population. According to different genetic studies on Egyptian Gaucher patients the homozygosity for the L444P mutation was the most common genotype, this type of mutation in the homozygous form is usually accompanied with chronic neuronopathic form of GD.

This mutation was found in high percent of cases in previous studies: El-Beshlawy et al. found that, out of 22 patients (59.1%) patients were homozygotes L444P [13,36]. Khalifa et al. detected among 48 GD patients, (66.7%) with homozygotes L444P, who exhibit GD III followed by type I (20.8%) while type II has the lowest frequency (12.5%) [6,10,32,37]. Tylki-Szymanska et al. mentioned that GD type III is mainly seen in Northern Europe, Egypt and East Asia [9].

Fateen et al. carried out a study on 34 Gaucher patients. This study showed that, GD III was the most common form followed by GD I and one patient with type II. The most common mutation was L444P detected in 28 alleles (43.75%), followed by IVS2+1G>A in 18 alleles (28%) and N370S in four (6.25%) alleles [38]. In a Tunisian study on 30 GD, 28 were classified type I GD and II patients were diagnosed types II and III [39].

In conclusion the prevalence of type GD III among Egyptian patients correlates well with the age of diagnosis of 97.5% of the patients which are diagnosed at a round the age of 2 years. The consanguinity rate among Egyptians was reported in many articles, Temtamy and Loutife stated that the consanguinity rate was 30%, in 1983 Hafze et al. stated that it was 28.9%. While Temtamy et al. reported that the consanguinity rate was 40% [40-42]. In the present study, the parental consanguinity was found in 3897 (76%) of the referred cases. While among the Gaucher diagnosed cases the parental consanguinity was found in 719 (81.6%), which is high and supported by previous studies among Egyptian GD patients, Ragab et al. (100%), El-Beshlawy et al. (68%), Khalifa et al. (88.8%) and Fateen et al. (45.5%) [36-38,43]. In a Syrian study on 19 GD patients, the consanguinity rate was 82%, which is very similar to our results [44]. The consanguinity among 425 Tunisian GD patients reached 341 (64.94%) [45]. Among 412 GD Brazilian patients, the

consanguinity rate reached 58% [46]. All these high consanguinity rates among GD patients match its autosomal recessive mode of inheritance. Although GD is an autosomal recessive disorders that affect both sexes equally, our results showed a difference between male patients' number (542) and female patients number (342) with a ratio (1.6: 1) this reflects traditions in Arab and Eastern countries; who give more care to males than females especially in rural areas where the consanguinity rate and metabolic disorders are high.

El-Beshlawy et al. showed, the male to female ratio among 21 GD patients was (1.2:1), Khalifa et al. stated that the ratio among 48 patients was (3.5:1), El-Morsy et al. found that, the ratio among 17 GD patients was (male: female 3.25:1), Fateen et al. showed that, the ratio in 34 GD patients was (male: female 1.6:1) [36-38,47,48]. However, Ragab et al., showed that, the ratio in 13 GD patients was (Male: Female 1: 1.16), and Tahia et al. revealed that, the ratio was (1.2:1) in a study that included 29 patients from Upper Egypt [43,49]. Figure 1 shows the male predominance in Gaucher diagnosed patients in this study, which agrees with all previously reported studies.

Similarly among 30 GD Tunisian patients the male:female ratio was (1.5:1) and in a study in India showed that among all the confirmed lysosomal storage disorders, the incidence in males was observed to be more common than in females, with male to female ratio (3.5:1) except for metachromatic leukodystrophy and mucopolysaccharidosis type IVA [34,50]. This study has no scientific explanation except the small number of the studied groups, which cannot give accurate estimate of the real ratios.

While among 412 Brazilian Gaucher patients, the females' number (237) was more than males (175) by ratio (1.35:1) which is different from our findings and the findings in other Arab countries [46]. The increased annual number of referred cases in the last four years of this study (380-551 cases) and the lower percent of positive GD cases (12%-15%) compared with the first years of study (37-146 cases) and the higher percent of diagnosed cases (21%-35%) reflects the increased awareness among the pediatricians about the diverse clinical features of GD and the availability of the specific investigations and treatment. Also, the need to facilitate early and accurate diagnosis of these conditions and to offer timely prenatal care. The most critical issue in this respect would be to sensitize and increase the Pediatricians awareness about the diverse clinical features of GD and the suitable investigations of this disorder when suspected. Increasing the awareness about this disorder in the general population is also desirable so that the parents too can pick up subtle features earlier in the course and avoid a delay in seeking medical advice. Also, to increase their awareness about the problems of consanguineous marriage and to combat this habit. Regarding all lysosomal disorders there must be always a dialog between the clinician and the lab. to pick up new variants of the diseases with uncommon manifestations.

The protocol for the diagnosis of GD starts with measurement of  $\beta$ -glucosidase in peripheral blood leukocytes, Baris et al. pointed that, the  $\beta$ -glucosidase activity in Gaucher patient was 0-15% and Cabrera-Ortiz et al. stated that cut of value of  $\beta$ -glucosidase was 30% in the studied cases confirmed by molecular study [11,51]. The cut off value in the present study was 30% of low normal value accompanied with a massive elevation of chitotriosidase, on average 78 times of high normal value of CT activity (Table 5).

107 patients (12.1%) showed a decrease in  $\beta$ -glucosidase activity to 70% of low normal value with an immense elevation of chitotriosidase

activity on average 120 times of high normal value, with normal sphingomyelinase enzyme activity.

Molecular study for diagnosed cases is an important step for further confirmation, which was done to some patients in several previous studies [37,38,48]. The benefits of conducting genotype analysis for patients with LSD are various. Besides sometimes confirming the diagnosis, it detects carriers within the affected families where enzyme analysis does not reliably detect heterozygous carriers because of the wide range of overlap between carriers and normal values [52]. It can be used for prenatal diagnosis but mainly it increases the phenotype prediction [52]. In case of GD where some mutations are known to be accompanied by neurologic manifestation like L444P and other mutations known to be mild ones like N370S.

The second step in the protocol is assessment of CT activity. As mentioned previously, all diagnosed GD cases showed a massive elevation in CT level than other LSDs [53]. The level of CT in this study was higher than the high normal value (80  $\mu\text{mol/L}$ ) with range (213-66700  $\mu\text{mol/l/h}$ ) and mean (7254.8  $\mu\text{mol/L}$ ,  $\pm$  20211.38  $\mu\text{mol/l/h}$ ). 103 patients out of 5128 (2%) showed a raise in CT with normal  $\beta$ -glucosidase activity. This led us to search for LSD and allowed us to establish other diagnoses, especially Niemann–Pick disease types A, B and C among the referred cases. Lo et al. reported a misdiagnosed NPD's case, who was diagnosed first as GD patient according to low  $\beta$ -glucosidase activity and increased chitotriosidase, then after a negative response to the therapy the case was re-evaluated and diagnosed as NPD patient with low sphingomyelinase activity and high chitotriosidase [54].

According to our similar experience through the years of study we always assess sphingomyelinase enzyme activity to all the referred GD patients. Still, some cases are diagnosed as GD. We attributed this to the poor quality of blood of these patients; which to unknown reason improves after ERT and by reassessment after few months of treatment a secured diagnosis of NPC was done. This raises the demands for new biomarkers to differentiate early between both diseases, Lobato et al. mentioned different biomarkers for different LSDs, glucosylsphingosine is as biomarker for GD, proposed by Rolfs et al. has emerged as a more effective alternative biomarker [21]. Their results show that the concentration of glucosylsphingosine in Gaucher samples was about 100 times higher than in healthy individuals and in patients with other LSDs. By using LC-MS/MS, the study achieved 100% specificity in identifying Gaucher patients.

Although, chitotriosidase activity is a reliable biomarker for the diagnosis and follow up of GD patients, there were 8 cases (1%) with zero CT activity. Boot et al. stated that about 4-5% of the population showed CT deficiency due to the presence of 24 bp duplication homozygous in exon 10 of the *CHIT1* gene [55]. So, another more specific biochemical marker is recommended for diagnose and follow-up in these cases, like (CCL18/PARC) or glucosylsphingosine. Misdiagnosis of GD and resulting diagnostic delays can occur in all types of GD patients, due to the vast heterogeneity in overall disease severity as well as differing patterns of organ involvement [56]. Almost all the patients attended the hematologist/oncologist during their search for diagnosis. Unfortunately, Gaucher disease is still under diagnosed due to the mild pre- adult form, mild signs and symptoms on one hand and the severe neurologic type GD II patients who die very early without being diagnosed.

As a result, the parents of the affected child lose out on timely genetic counselling, and the advantage of prenatal diagnosis. This puts the couple on the risk of having another affected child, thereby

increasing the load of these diseases even further. In the present study, 33 families (0.77%) had history of an affected sibling notably in the first years of study, but due to lack of awareness they unfortunately delivered another affected sibling. In the recent years all diagnosed families' sought prenatal diagnosis. Although the awareness is now much better than in the nineties but still increasing the awareness and training the pediatricians about the diverse clinical features of GD and the suitable investigations for this disorder are a critical issue for early and accurate diagnosis.

Moreover, increasing the awareness about this disorder in the general population is desirable and mainly in families with affected siblings' consanguineous marriages so, the parents can pick up subtle features earlier and avoid a delay in seeking medical advice. The basic care for GD is macrophage-directed enzyme replacement therapy ERT in which the defective  $\beta$ -glucosidase is supplemented with recombinant glucocerebrosidase, administered by intravenous infusions usually every 2 weeks [57]. As expected, hepatosplenomegaly and hematological manifestations were markedly improved. In 1999, Project HOPE began to implement the Gaucher Disease Initiative in Egypt in partnership with Genzyme Corporation, through donation of more than 30 million units of Cerezyme (imiglucerase for injection). 282 is the total number of cases that receive therapy in Egypt till now. According to the data we had in this study, 28 patients (10%) are regularly doing follow up by CT enzyme activity. Their CT activity has decreased from mean 5185  $\mu\text{mol/l/h}$  to mean 904  $\mu\text{mol/l/h}$ , the time for follow-up is variable between patients. The awareness of follow up of the treatment by CT activity must be increased among the patients and treating physicians. By this regular follow up the ERT can be tuned; which is very important for such very an expensive medication. In the general population the prevalence of GD 3 is 1:100.000 so the total number of diagnosed Gaucher patients in Egypt should be 1000 cases according to the calculation using the prevalence and the total number of the population in Egypt (100.000.000), that means the difference between what we diagnosed over 25 years and what we predict is small 1000-881=119 cases around Egypt [58,59] which lead us to think that the prevalence in Egypt may be higher than 1:100.000.

## Conclusion

Lysosomal disorders are rare, inherited disorders that impact metabolic processes resulting from dysfunction or loss of function of lysosomal enzymes. Early diagnosis is critical in determining treatment options for patients, and there are some evidences that starting treatment early mostly result in a better outcome. The purpose is to review the molecular and biologic principles behind early treatment of lysosomal disorders with a focus on fabry and pompe diseases. The panel of experts in lysosomal diseases will explore the opportunities and challenges of starting treatment early before irreversible organ damage occurs.

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