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Case Report

The role of glucosylsphingosine as an early indicator of disease progression in early symptomatic type 1 Gaucher disease

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ABSTRACT

Gaucher disease (GD), a lysosomal storage disorder caused by β-glucocerebrosidase deficiency, results in the accumulation of glucosylceramide and glucosylsphingosine. Glucosylsphingosine has emerged as a sensitive and specific biomarker for GD and treatment response. However, limited information exists on its role in guiding treatment decisions in pre-symptomatic patients identified at birth or due to a positive family history. We present two pediatric patients with GD1 and highlight the utility of glucosylsphingosine monitoring in guiding treatment initiation.

1. Introduction

Gaucher disease (GD, MIM #230800), the most common lysosomal storage disorder, is caused by biallelic pathogenic variants in *GBA*, resulting in enzymatic deficiency of β-glucocerebrosidase (EC 3.2. 1.45) [1]. The β-glucocerebrosidase enzyme catalyzes the metabolism of glycosphingolipids in the lysosomal globoside degradation pathway and enzymatic deficiency results in an intra-lysosomal accumulation of glucosylceramide [2,3]. As glucosylceramide accumulates in lysosomal macrophages of the spleen, liver, bone marrow, and occasionally the lungs, it undergoes deacylation by acid ceramidase to form glucosylsphingosine (lyso-Gb_1), a sensitive biomarker for GD [4,5]. The accumulation of these substrates can lead to progressive, debilitating disease with multi-system involvement [6].

Type 1 GD is the most common form in the United States and Europe with an estimated disease prevalence of 1 in 40,000 to 60,000 individuals, with an increased prevalence in individuals of Ashkenazi Jewish descent [6,7]. Phenotypically, type 1 GD is distinguished from types 2 and 3 by a lack of central nervous system involvement. The most prevalent pathogenic variant in patients with type 1 GD is the c.1226A > G, p.N409S (NM_000157.4) allele, which can be attributed to a founder effect in the Ashkenazi Jewish population. Individuals homozygous for this variant present with a variable clinical presentation ranging from early-onset disease in childhood to onset in the sixth decade of life [8]. Symptoms may include hepatosplenomegaly, anemia, thrombocytopenia, growth delay, and bone or pulmonary involvement [7,9,10]. Targeted treatment in the form of enzyme replacement therapy (ERT) and substrate reduction therapy (for adults) are available and effective at reversing disease symptoms [11–13].

Disease progression is monitored in patients with GD via analysis of blood counts and chitotriosidase (CHITO) activity [14] at baseline and every 3 to 6 months or 12 months for ERT treated and untreated patients, respectively [15]. Visceral and skeletal involvement is assessed at diagnosis and every 6 to 12 months for treated and untreated patients, respectively, using magnetic resonance imaging (MRI) and ultrasound (US) for visceral assessment and MRI of the femora, spine and symptomatic sites for skeletal assessment [16]. Furthermore, in addition to assessment of bone marrow infiltration, bone mineral density is assessed using dual energy x-ray absorptiometry (DEXA) [17]. More recently, lyso-Gb_1 has shown to be a useful and reliable biomarker for the diagnosis and monitoring of treated and untreated patients with GD [14,18,19].

Here we report the clinical, biochemical, and molecular findings of two unrelated pediatric patients identified due to high risk screening with type 1 GD. Both patients were followed closely and treatment was initiated based on clinical evidence of disease progression and rising biomarker levels. We highlight the clinical utility of lyso-Gb_1 in the decision-making tree and also its benefits in evaluating treatment

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response.

2. Methods

Medical records were reviewed for two unrelated pediatric patients diagnosed with type 1 GD with lyso-Gb_1 data available. The two patients were followed at different institutions and managed utilizing standard surveillance and treatment guidelines. Review of the patients' medical records and clinical parameters was performed at each institution by the respective treating clinical team. The first three samples for lyso-Gb_1 analysis from patient 1 were obtained from a repository study 'Genetic Disease Repository for Blood, Urine and Tissue' (IRB# Pro00007612) and lyso-Gb_1 analysis was performed under a research study (IRB# Pro00088186). Both studies were approved by the Duke University Health System Institutional Review Board.

Plasma lyso-Gb_1 analysis for Patient 1 was performed according to a previously published method [20] by Duke University Health System's CLIA/CAP certified Biochemical Genetics Laboratory. CHITO analysis was sent to LabCorp, a CLIA/CAP certified laboratory.

Plasma lyso-Gb_1 analysis and CHITO activity for Patient 2 were performed by Sema4, DBA Mount Sinai Genomics Inc., a CLIA/CAP certified laboratory. In brief, lyso-Gb_1 analysis was performed by liquid-chromatography tandem mass-spectrometry (LC-MS/MS) and CHITO activity was measured by a fluorometric method using artificial 4-MU-β-*D*-triacetylchitotriosidase substrate.

3. Case reports

3.1. Patient 1

Patient 1 is a 7-year-old Caucasian male homozygous for the *GBA* p.N409S pathogenic variant, diagnosed prenatally via amniocentesis after his parents were identified as GD carriers on prenatal carrier screening. He was first evaluated at 6 months of age and established care at Duke University at 3 years of age with follow-up appointments approximately every 6 months, with his most recent examination at 7.5 years. Height, weight, and head circumference trends have always been appropriate for age. Initial abdominal MRI at 4.0 years of age revealed persistent hepatosplenomegaly (Table 1). DEXA bone density evaluation at 6.4 years of age was normal with a total spine *Z*-score of 1.6 and total body Z-score of 1.9.

Hemoglobin and platelet counts have always been within normal limits; however, marked elevations of lyso-Gb_1 and CHITO were consistently observed (Fig. 1A). Lyso-Gb_1 concentration was elevated at 45.8 nmol/L (NL: <1.9) at first assessment and remained moderately elevated with a maximum observed concentration of 97.5 nmol/L. Similar trends were observed for CHITO with concentrations as low as 789 mmol/h/ml (NL: 5.1–120) at first collection and as high as 1637 mmol/h/ml at the most recent collection prior to treatment initiation. At no point was there a history of bone crises, lytic lesions, avascular necrosis, or pathological fractures. There were no complaints of pain, fatigue, increased bleeding, or developmental delays; however, in view of the persistent hepatosplenomegaly and rising biomarker levels, treatment with ERT was recommended. The patient began treatment at 7.4 years of age with velaglucerase alfa (VPRIV®) at the standard dose of 60 units/kg every other week. After 3 months of ERT, biomarker values showed a marked reduction.

Table 1
Patient organ volume and BMI.

	Age (yrs)	Liver		Spleen		BMI (kg/m2)
		Volume (ml)	MN	Volume (ml)	MN	
Patient 1	3.9	586	1.2	142	3.7	16.9
	6.1	770	1.3	181	3.8	15.9
	7.0	873	1.2	202	3.5	17.2
Patient 2	6.5	1028	1.9	139	3.2	15.4
	7.3	1189	2.1	159	3.5	14.6
	8.4	1023	1.7	128	2.6	15.2

3.2. Patient 2

Patient 2 is a 9-year-old male of Ashkenazi Jewish descent with a positive family history of GD, homozygous for the *GBA* p.N409S pathogenic variant. Diagnosis was made at 5 years of age via targeted *GBA* genetic testing and care established at the Icahn School of Medicine at Mount Sinai with follow-up appointments approximately every 12 months. Gross/fine motor delays and speech delays were observed upon initial examination at 5 years of age, which resolved with physical and occupational therapy. Liver and spleen volumes as obtained via abdominal ultrasound showed mild organomegaly from 6.5 years of age (Table 1). Most recent DEXA bone density evaluation performed at 9 years of age showed a *Z*-score of −2.4 and − 1.1 at the hip and spine, respectively. At 9 years of age, the patient noted joint pain in knees, feet and hands and reported easy bruising and fatigue.

Hemoglobin levels consistently measured within the normal range while platelets had dropped to borderline low at 8.4 years of age. Within one and a half years, biomarker levels nearly doubled, increasing from: 122.6 to 211.6 nmol/L (NL: 0–2.17) for lyso-Gb_1 while CHITO values increased slightly from 4680 to 5000 mmol/h/ml (NL: 5.1–120). There was no history of bone crises, lytic lesions, avascular necrosis, or pathological fractures; however, as the patient exhibited evidence of disease burden with increasing levels of biomarkers including lyso-Gb_1 and CHITO, ERT was recommended and initiated at 9.2 years of age. After 5 months of treatment with Imiglucerase (Cerezyme®) at the standard dose of 60 units/kg every other week, a marked reduction in biomarker levels was observed (Fig. 1B).

4. Discussion

Increasing numbers of pre-symptomatic children with GD are being identified due to widely-accessible carrier screening and incorporation of GD into newborn screening. As these individuals are being identified prior to disease manifestation, it is important that effective disease monitoring is established to determine the optimal time to begin treatment, and thus prevent long-term, irreversible damage.

Although the p.N409S pathogenic *GBA* variant is a common pathogenic variant in type 1 GD [21], individuals who are homozygous for this allele exhibit a high degree of phenotypic heterogeneity [22]. While some individuals with this genotype may present with mild symptoms and/or remain asymptomatic for decades [9], others may present in childhood with severe clinical manifestations including hepatosplenomegaly, pancytopenia, growth delay, and bone and lung involvement [17,23]. Therefore, disease monitoring and initiation of treatment must be tailored to the individual based on their specific disease course.

Guidelines for the evaluation and management of patients with GD recommend a comprehensive evaluation of all disease domains to establish baseline disease characteristics to monitor for treatment initiation [24]. In addition, guidelines for the management of asymptomatic children with GD recommend annual examinations for individuals with the p.N409S variant and more frequent monitoring for individuals with variants known to result in a more severe clinical presentation [15]. Yang et al. reported the clinical, laboratory, and imaging data of 38 pre-symptomatic children with type 1 GD diagnosed after parental carrier screening, and concluded that disease progression could be effectively monitored via laboratory studies including CBC and CHITO activity at each visit, assessment of organ volumes every 1–2 years via abdominal ultrasound or MRI, and assessment of bone mineral density via DEXA every 2 years. Treatment was initiated when growth parameters were not met, osteopenia was present as evidenced on DEXA

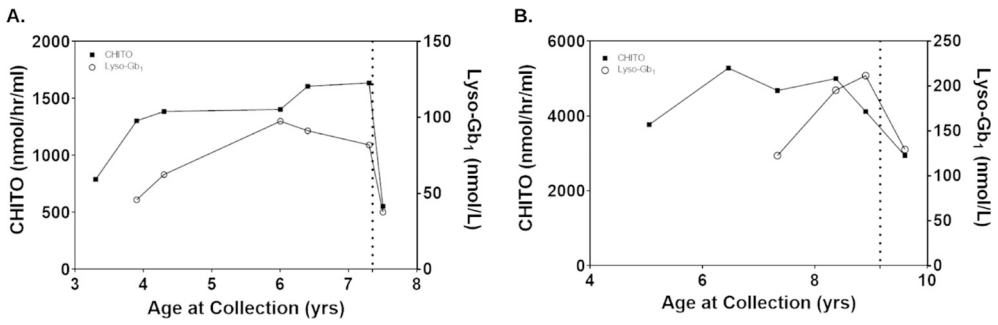

Fig. 1. Biomarker values in (A) Patient 1 and (B) Patient 2. Vertical dotted line denotes start of treatment with ERT.

scan, organomegaly was present, and/or an increasing trend of CHITO was observed [25].

CHITO has been shown to be a valuable marker in the assessment and monitoring of patients with GD [12,26,27]; however, CHITO analysis will be unreliable in ~6% and ~ 33% of the general population who are homozygous or heterozygous, respectively, for a benign modifier variant in *CHIT1* [28,29], thus resulting in a barrier to monitoring disease progression in a significant proportion of individuals. In place of CHITO, some physicians may choose to monitor therapeutic response with periodic assessment of ACE and TRAP, which have a high degree of correlation with CHITO levels; however, as they are not specific to GD, they are not always utilized in the clinical assessment of patients with GD [29] and lyso-Gb_1 analysis allows for improved disease monitoring in these patient.

More recently, lyso-Gb_1 has been shown to be a key biomarker of GD and its response to treatment [4,18,19,30–32] and several analytical methods have been developed to measure lyso-Gb_1 in plasma, urine, and dried blood spots [33–37]. The development of clinical assays for measurement of lyso-Gb_1, a sensitive biomarker for GD, has the potential for major clinical utility in monitoring disease progression in all patients with GD; however, there is increased utility of this biomarker in patients such as those described herein that were diagnosed prenatally or in early childhood, to determine when to initiate treatment with ERT. While Patient 1 did not outwardly exhibit signs of disease such as pain, fracture, or bleeding, moderate elevations of lyso-Gb_1 in the setting of persistent hepatosplenomegaly was evidence of disease and allowed the physician and family to make the decision to initiate treatment. For Patient 2, elevated lyso-Gb_1 in the setting of pain, low bone density and borderline low platelets provides supportive evidence for the decision to initiate treatment. The elevated lyso-Gb_1 together with the elevated CHITO observed in both patients strengthens the evidence of disease progression; however, additional data from larger cohorts would be beneficial to show the clinical utility of lyso-Gb_1. As both patients continue to receive ERT for GD, lyso-Gb_1 will remain an important tool to monitor effectiveness of treatment until organ volume and bone density are assessed.

5. Conclusion

The addition of lyso-Gb_1 in the clinical assessment of two pediatric patients with type 1 GD diagnosed pre-symptomatically allowed accurate monitoring of disease progression and determination of ERT initiation. The persistent elevations of lyso-Gb_1 together with the persistent elevations of CHITO was an important factor in the decision to initiate treatment. Upon treatment initiation with ERT, a marked reduction of biomarker levels was observed, highlighting the utility of this biomarker in treatment monitoring. Integration of lyso-Gb_1 would be a beneficial addition to the routine monitoring guidelines for patients with GD to inform treatment decisions and other clinical determinations as well as treatment monitoring.

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