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Evaluating biomarkers for diagnosis and treatment monitoring in Gaucher Disease

Gaucher Hastalığında tanı ve tedavi izleminde biyobelirteçlerin değerlendirilmesi

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ABSTRACT

Aim: The primary goal of this study is to explore the impact of consistent treatment on key disease marker, Lyso-Gb1. Additionally, this research aims to evaluate the influence of splenectomy on Lyso-gb1 concentrations within the patient group.

Materials and Methods: 37 patients diagnosed with GD were categorized based on treatment compliance into three groups: 28 in the regular treatment group, who consistently followed their treatment; 6 in the irregular treatment group, with inconsistent treatment adherence; and 3 in the untreated group. A control group of 33 healthy individuals without GD was also included. Enzyme replacement therapy was utilized as the treatment regimen. The analysis of Lyso-Gb1 levels was performed using liquid chromatography coupled with tandem mass spectrometry, ensuring high precision in measurement.

Results: Lyso-Gb1 levels were significantly higher in GD patients compared to the healthy control group ($p < 0.05$), affirming its potential as a specific biomarker. Treatment was associated with a reduction in Lyso-Gb1 levels ($p < 0.05$). No significant difference in Lyso-Gb1 levels was observed between treated patients with Type 1 and Type 3 GD ($p > 0.05$). Notably, patients who underwent splenectomy exhibited significantly higher Lyso-Gb1 levels than those who did not ($p < 0.05$).

Conclusion: Our findings support the utility of Lyso-Gb1 as a specific biomarker for GD. While pre-treatment Lyso-Gb1 levels in the treated group remain unknown, our results underscore the need for larger, longitudinal studies to further elucidate Lyso-Gb1's role in monitoring disease progression and treatment efficacy in GD.

Keywords: Biomarker, Gaucher disease, glucosylsphingosine, Lyso-Gb1, lysosomal storage disorders.

ÖZ

Amaç: *Bu çalışmanın temel amacı düzenli tedavinin anahtar hastalık belirteci Lyso-Gb1 üzerindeki etkisini araştırmaktır. Ayrıca bu araştırma, hasta grubunda splenektominin Lyso-Gb1 konsantrasyonları üzerindeki etkisini değerlendirmeyi amaçlamaktadır.*

Gereç ve Yöntem: *Gaucher Hastalığı (GH) tanısı alan 37 hasta tedavi durumuna göre üç gruba ayrıldı: Tedavilerini tutarlı bir şekilde takip eden düzenli tedavi grubunda 28; düzensiz tedavi grubunda 6 ve henüz tedavi almayan grupta 3 hasta mevcuttu. GH olmayan 33 sağlıklı bireyden oluşan bir kontrol grubu da dahil edildi. Tedavi rejimi olarak enzim replasman tedavisi kullanıldı. Lyso-Gb1 seviyelerinin analizi, ölçümde yüksek hassasiyet sağlayan tandem kütle spektrometresi ile birleştirilmiş sıvı kromatografisi kullanılarak gerçekleştirildi.*

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Bulgular: *Lyso-Gb1 seviyeleri Gaucher hastalarında sağlıklı kontrol grubuyla karşılaştırıldığında anlamlı derecede yüksekti (p<0.05), bu da bunun spesifik bir biyobelirteç olma potansiyelini doğruluyor. Tedavi, Lyso-Gb1 seviyelerinde bir azalma ile ilişkilendirildi (p<0.05). Tedavi edilen Tip 1 ve Tip 3 GH arasında Lyso-Gb1 düzeyleri açısından anlamlı bir fark gözlenmedi (p>0,05). Özellikle splenektomi yapılan hastalarda, yapılmayanlara göre anlamlı derecede daha yüksek Lyso-Gb1 seviyeleri sergilendi (p<0.05).*

Sonuç: *Bulgularımız Lyso-Gb1'in GH için spesifik bir biyobelirteç olarak kullanımını desteklemektedir. Tedavi edilen grupta tedavi öncesi Lyso-Gb1 seviyeleri bilinmemekle birlikte, sonuçlarımız Lyso-Gb1'in GD'de hastalığın ilerlemesini ve tedavi etkinliğini izlemedeki rolünü daha fazla aydınlatmak için daha büyük, boylamsal çalışmalara olan ihtiyacın altını çiziyor.*

Anahtar Sözcükler: *Biyobelirteç, Gaucher hastalığı, glukozilsfingozin, Lyso-Gb1, lizozomal depo hastalıkları.*

INTRODUCTION

Gaucher Disease (GD) stands as the most common lysosomal storage disorder, attributable to biallelic mutations in the GBA gene. These mutations disrupt the normal function of the β-glucocerebrosidase enzyme, essential for cleaving glucosylceramide into glucose and ceramide in unaffected individuals (1). Classified based on neurological involvement, GD manifests in three distinct forms: Type I (T1GD), Type II (T2GD), and Type III (T3GD) (2). Although the majority of Turkish patients have been reported to have T1GD, there are regional differences in Turkey (3-5). As the burden of GD is profound, the patients often experience severe symptoms across multiple somatic organs. However, these effects can be mitigated through treatments such as enzyme replacement therapy (ERT) or substrate reduction therapy (SRT), which have shown efficacy, particularly in the management of T1GD and T3GD (6).

Diagnosis typically follows clinical suspicion, with initial assessments focusing on reduced acid β-glucocerebrosidase activity in blood and/or tissues, and is confirmed through genetic analysis identifying pathogenic variants in the GBA gene (2). Traditional biomarkers for GD, including acid phosphatase, angiotensin-converting enzyme, ferritin, chitotriosidase, and chemokine ligand 18, have been employed for assessment and follow-up. However, their lack of disease specificity and sensitivity has been a significant limitation, often resulting in diagnostic uncertainty. The discovery of glucosylsphingosine (Lyso-Gb1), a deacylated derivative of glucosylceramide, has advanced the search for a definitive biomarker. Lyso-gb1 has been consistently elevated in individuals with GD, positioning it as a potential key indicator for diagnosing the disorder. Nonetheless, there is still a need for studies focusing on the specificity of Lyso-Gb1 in reflecting the presence of GD and its fluctuation in response to therapeutic interventions (7).

Given the necessity for tracking disease progression and gauging response to treatments, our research is dedicated to affirming the biomarker's clinical significance. Our investigation presents a thorough comparative study of the levels of several biomarkers in GD patients relative to healthy individuals. By employing both longitudinal and cross-sectional methods, we investigated the effects of treatment regularity on a variety of essential disease indicators, with an emphasis on Lyso-Gb1. The treatment regimen consisted of ERT. The indicators included measurements of Lyso-Gb1, platelet counts, hemoglobin, ferritin, and chitotriosidase levels. Furthermore, we assessed the effect of splenectomy on Lyso-GHb1 concentrations among the patient cohort.

Exploring the complexities of GD through the lens of our dataset reveals intriguing avenues for investigation, particularly in understanding how genotypic variations influence the clinical severity and treatment outcomes of this condition. One area of focus is the relationship between specific genotypes and the clinical manifestations of GD. It is hypothesized that patients exhibiting certain genotypic classifications, such as being homozygous for allele N370S, may present milder clinical symptoms and show more significant improvements in blood biomarkers following treatment compared to those with other genetic backgrounds. Furthermore, the timing of diagnosis poses another critical factor in the management and prognosis of the disease. It is posited that an early diagnosis could lead to

more effective disease management and better clinical outcomes, as evidenced by improvements in key blood biomarkers. Moreover, this dataset prompts an examination of potential gender-specific differences in both the clinical presentation of GD and the response to treatment, suggesting that male and female patients may experience and respond to the disease in distinct ways. Additionally, the study aims to delve into how specific genotypes correlate with baseline levels of certain biomarkers, such as chitotriosidase and Lyso-Gb1, and how these levels change in response to treatment across different genotypes. This multifaceted research approach not only seeks to unravel the genetic underpinnings of GD but also aims to enhance our understanding of its clinical implications and inform more tailored treatment strategies.

MATERIALS and METHODS

Categorization of Study Groups

The present case-control study encompassed seventy participants (Figure-1). The participants were divided into a control group (n=33) and a case group (n=37). The latter, comprising GD patients, was subdivided into four categories based on treatment adherence. These included: patients with Type 1 GD (T1GD) and Type 3 GD (T3GD) who received continuous treatment (termed the regular T1GD and regular T3GD groups, respectively), patients who had discontinued treatment for six months or longer, constituting the irregular T1GD group; and untreated patients, referred to hereafter as 'native' patients. This segmentation facilitated a detailed analysis aimed at identifying variations in clinical and biochemical parameters among different treatment statuses within the GD cohort. Of note, the case group included 32 patients with T1GD and five with T3GD.

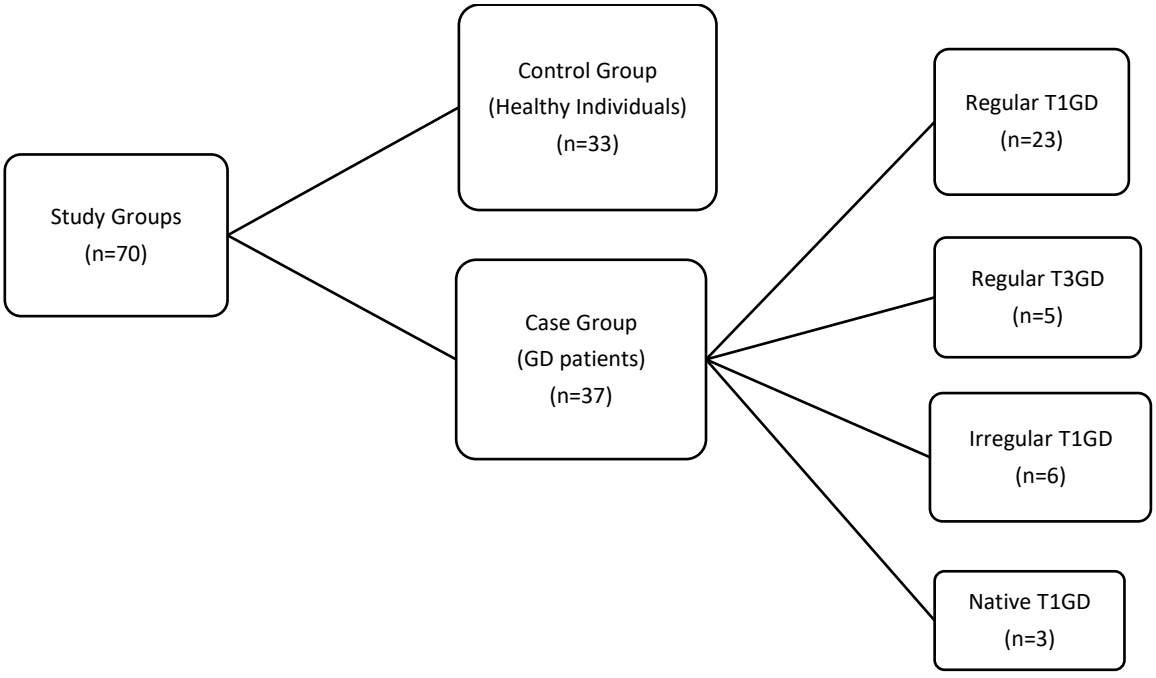

Figure-1. Categorization of study groups. *N: Number of individuals; GD: Gaucher's Disease; T1GD: Type1 Gaucher's Disease; T3GD: Type3 Gaucher's Disease.*

Demographic, Clinical and Laboratory Characteristics

The study examined various demographic, clinical, and laboratory parameters, including type of GD (T1GD or T3GD), gender, age at diagnosis age at treatment, genotypic classification based on alleles 1 and 2, type of treatment (imiglucerase or taliglucerase-α) and dosage of treatment (30 or 60 U/kg/q2wks; low or high dose, respectively), the occurrence of surgical intervention (splenectomy), the presence of comorbid diseases (none vs. one or more), and blood ferritin (F) level-1 and -2, blood hemoglobin (HB) level-1 and -2, white blood cells (WBCs)

count-1 and -2, blood platelets (PLT) count-1 and -2, blood chitotriosidase (C) level-1 and -2, blood lyso-Gb1 (L) level-1 and -2.

These parameters were systematically assessed to understand their interrelations and correlations with several outcomes. The second measurements were reported six months later than the first measurements.

Measurement of Plasma Lyso-Gb1 Levels by LC-MS/MS

In the study, high-purity glucosylsphingosine and lyso-lactosylceramide were used as primary and internal standards, respectively, sourced from reputable suppliers. The analytical process involved ultra-performance liquid chromatography (UPLC) using a Waters ACQUITY system with a specific methanol and formic acid mobile phase protocol. Mass spectrometry was performed using a Waters XEVO TQD system, employing enhanced ionization conditions and gas flows for precise quantification. The method developed by Ouyang et al. Has been modified and used (8). 100 µL of Lyso GB-2 (IS) (50 ng/mL, prepared in methanol) and 1 mL of methanol/acetone (V/V, 1/1) were added to 50 µL of plasma and vortexed for 30 seconds. The mixture is then centrifuged at 14000 g for 10 minutes at +4 °C. The supernatant is transferred to an LC-MS MS plate and evaporated under nitrogen gas and then reconstituted with 100 µL of methanol. After having vortex-mixed thoroughly for 30 seconds, a volume of 2 µL was injected into the LC-MS/MS system.

Statistical Analysis

Descriptive statistics such as mean, standard deviation, median, minimum and maximum values, as well as frequency and percentages, were calculated. The Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to assess the normal distribution of the variables. Comparative analyses were performed using the Mann-Whitney U test for quantitative data, the Wilcoxon test for repeated measures, and the Chi-square test for qualitative data. Statistical analyses were conducted using SPSS version 25.0. Results with $p<0.05$ were considered as statistically significant.

RESULTS

Comparative Analysis of Lyso-Gb1 Levels Between Multiple Groups

The difference in Lyso-gb1 results between the healthy individuals (control) (n=33) and GD patients (case) (n=37) is statistically significant (Mann-Whitney U test, $p<0.001$) (Figure-2, Figure-3).

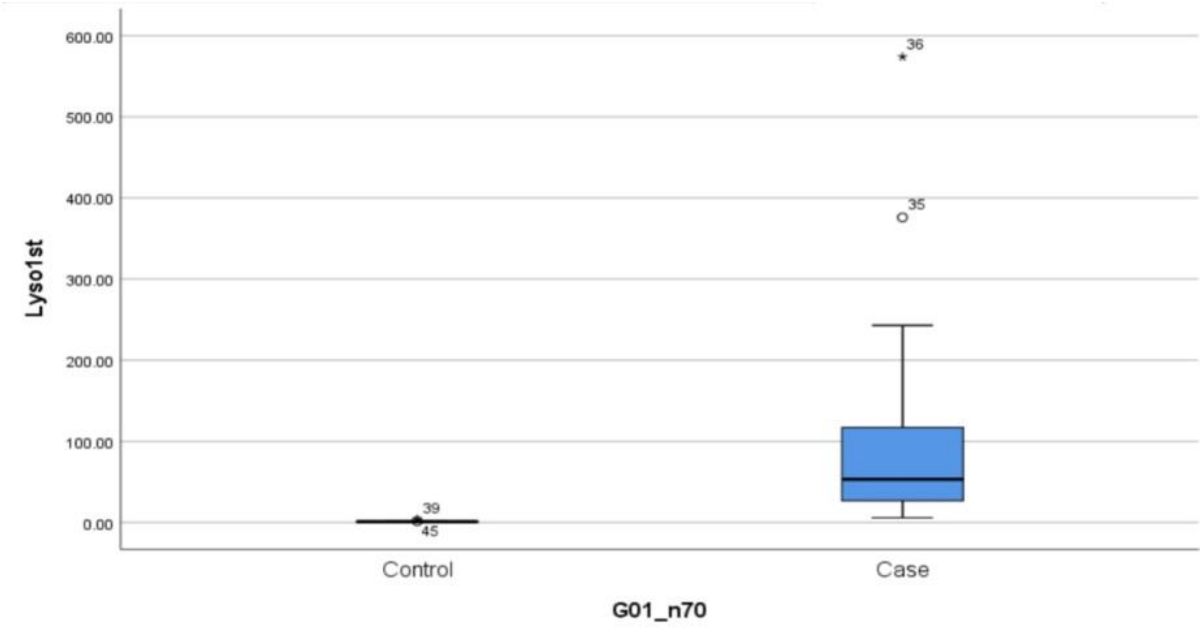

Figure-2. Comparison of Lyso-Gb1 levels between healthy individuals (control) and GD patients (case).

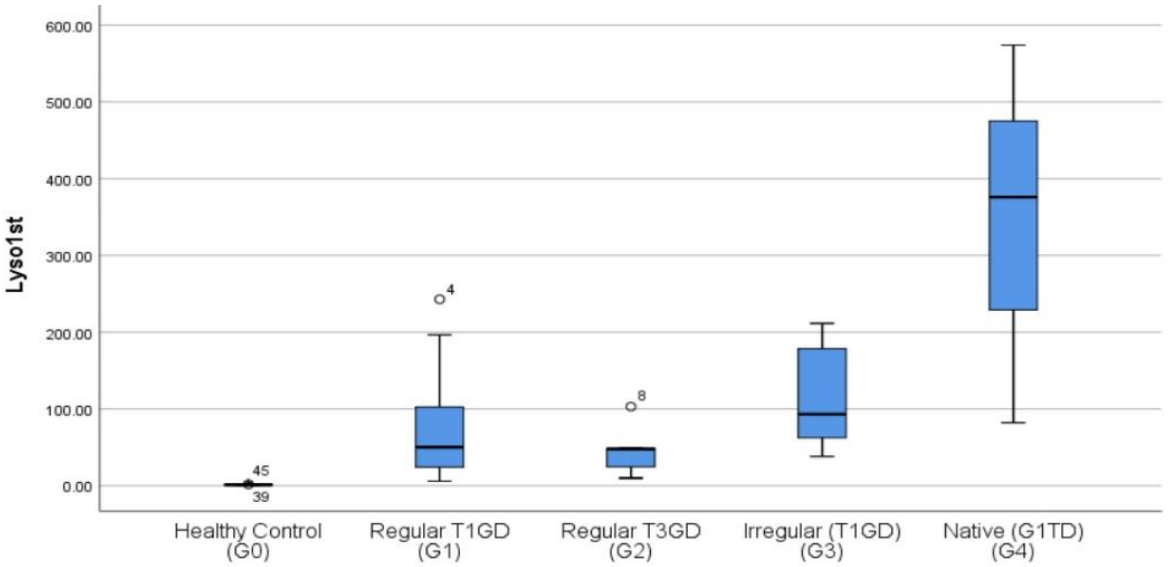

Figure-3. Comparative analysis of Lyso-Gb1 levels between multiple groups.

Comparative Analysis of Demographic and Clinical Features

Table-1 presents a comparative analysis of demographic and clinical features across study groups concerning treatment adherence. The groups are categorized by disease type, gender, surgical intervention, comorbid diseases, and genotype classification. The treatment adherence is divided into regular and irregular.

Table-1 shows that 71.4% of girls (20 out of 28) adhered regularly to treatment, whereas 28.6% of boys (8 out of 28) did so. In contrast, a higher proportion of boys showed irregular adherence (66.7%, 4 out of 6) compared to girls (33.3%, 2 out of 6). The p-value for gender is 0.076, which is above the conventional threshold of 0.05 for statistical significance. This suggests that there is no statistically significant difference in treatment adherence between boys and girls in this study.

In terms of disease types, T1GD and T3GD are compared. Of the patients with T1GD, 82.1% (23 out of 28) had regular adherence, and all patients with T1GD (6 out of 6) who were non-adherent were irregular. There were no patients with T3GD who were irregular in their adherence. The p-value here is 0.559, indicating no statistically significant difference between the disease types T1GD and T3GD in terms of treatment adherence.

When looking at surgical intervention, 25% of splenectomized patients (7 out of 28) were regular in their adherence, compared to 75% of non-splenectomized patients (21 out of 28). For irregular adherence, 16.7% were splenectomized (1 out of 6), and 83.3% were non-splenectomized (5 out of 6). The p-value is 1, signifying no statistical significance in adherence to treatment between splenectomized and non-splenectomized groups.

In brief, the data from Table-1 suggests no statistically significant association between treatment adherence and the demographic or clinical features studied, given the p-values are all above the standard threshold of 0.05. This means that the differences observed in adherence rates across gender, disease type, and surgical intervention are not statistically significant and could be due to chance.

The first measurement Lyso-Gb1 value in the splenectomy group was significantly ($p < 0.05$) higher than the non-splenectomy group. In the splenectomy group, the second measurement Lyso-Gb1 value did not show a significant change ($p>0.05$) compared to the first measurement. In the group without splenectomy, the second measurement Lyso-Gb1 value did not show a significant change ($p>0.05$) compared to the first measurement. The amount of Lyso-Gb1 change in the second measurement did not differ significantly ($p > 0.05$) in the groups with and without splenectomy (Table-2).

Table-1. Comparison of demographic, clinical, and genotype characteristics of GD patients.

		Gender		SI		CoM		Genotype Classification		
		Female	Male	NS	S	Unknown	One or more	A N370S +/+	B N370S +/- or -/+	C Non-N370S
Regular T1GD	Count	18	5	16	7	17	6	8_a	$14_{a, b}$	1_b
	Expected Count	16.1	6.9	18.0	5.0	16.2	6.8	5.6	13.7	3.7
	% within Group	78	22	70	30	74	26	35	61	4
Regular T3GD	Count	2	3	5	0	3	2	0_a	0_a	5_b
	Expected Count	3.5	1.5	3.9	1.1	3.5	1.5	1.2	3.0	0.8
	% within Group	40	60	100	0	60	40	0	0	100
Irregular T1GD	Count	2	4	5	1	3	3	0	6	0
	Expected Count	4.2	1.8	4.7	1.3	4.2	1.8	1.5	3.6	1.0
	% within Group	33	67	83	17	50	50	0	100	0
Native T1GD	Count	2	1	3	0	3	0	1	2	0
	Expected Count	2.1	0.9	2.4	0.6	2.1	0.9	0.7	1.8	0.5
	% within Group	67	33	100	0	100	0	33	67	0

*SI: Surgical Intervention, NS: Non-splenectomized, S: Splenectomized, CoM: Comorbidity. *Chi-square (X^2) test (Fisher's Exact: Gender p=0.103; Surgical Intervention p=0.468; Comorbidity p=0.488; Genotype Classification: p<0.001)*

Table-2. Comparison of laboratory test levels according to splenectomy.

Variable	Splenectomy (-)			Splenectomy (+)			p	
	Mean±sd		Median	Mean±sd		Median		
Lyso-Gb1								
First	43.8 ± 36.9		30.9	120.3 ± 77.0		117.1	***0.008***	m
Second	33.0 ± 36.6		21.8	111.3 ± 43.8		129.1	***0.001***	m
Variation	-5.3 ± 22.8		-2.6	-9.0 ± 50.5		2.0	0.545	m
Variation in groups p	0.145		w	1.000		w		

m Mann-Whitney u test / w Wilcoxon test

Pharmacological Treatment Distribution in Gaucher's Disease Patients

In the treatment cohort for GD, a total of thirty individuals received imiglucerase, while another four were administered taliglucerase-alfa. ERT was dosed between 30 and 60 units per kilogram biweekly (U/kg/q2wk) (Table-3).

The therapy was continued for an average duration of 26.8 months, with a standard deviation of 13.5 months, ranging from 6 to 36 months in length. Throughout the treatment period, no serious adverse effects were reported.

Table-3. Distribution of GD patients according to treatment regimens.

Treatment Regimen		Regular		Irregular		Total (N)
Agent Name	**Dose***	**N**	**Ratio (%)**	**N**	**Ratio (%)**	
Imiglucerase	30	12	71	5	23	17
	60	13	100	0	0	13
Taliglucerase-α	30	1	50	1	50	2
	60	2	100	0	0	2

**U/kg/q2wk*

Table-4. Results of clinical and laboratory features across GD patient groups according to treatment adherence.

Treatment adherence		Regular		Irregular		p	
Variable		**Mean±sd/n-%**	**Median**	**Mean±sd/n-%**	**Median**		
Age of diagnosis		17.4 ± 17.7	11.5	23.7 ± 15.9	20.5	0.222	[m]
Gender	Girl	20 71.4%		2 33.3%		0.076	[X²]
	Boy	8 28.6%		4 66.7%			
Gaucher	Type I	23 82.1%		6 100%			[X²]
	Type III	5 17.9%		0 0.0%			
Splenectomy	(-)	21 75.0%		5 83.3%		1.000	[X²]
	(+)	7 25.0%		1 16.7%			
Lyso-Gb1		63.7 ± 58.9	48.7	112.7 ± 67.7	93.0	**0.031**	[m]
WBC		6680 ± 2512	6010	8200 ± 2247	7020	0.140	[m]
PLT (x10³)		222.7 ± 61.0	231.5	171.0 ± 25.2	158.0	**0.026**	[m]
Hemoglobin		13.0 ± 1.4	12.7	14.2 ± 0.8	14.1	0.072	[m]
Ferritin		230.8 ± 333.3	92.5	386.8 ± 342.4	274.0	0.118	[m]
Chitoriosidase		49.4 ± 38.7	51.0	97.0 ± 10.4	91.0	**0.041**	[m]

[m] Mann-whitney u test/ [X²] Chi-square test

Tablo-5. Comparison of laboratory test levels according to *g*enotype classification.

	Genotype A N370S +/+ (n=9)		Genotype B N370S +/- or -/+ (n=19)		Genotype C Non-N370S (n=6)		p
	Mean±sd	**Median**	**Mean±sd**	**Median**	**Mean±sd**	**Median**	
Lyso-Gb1	56.66 ± 73.57	28.35	85.75 ± 63.66	28.35	48.93 ± 32.16	48.17	0.2485 [k]
WBC	6421 ± 2675	5290	7033 ± 2212	6730	7340 ± 3578	6590	0.6515 [k]
PLT (x10³)	214.113 ± 73.28	227.500	199.89 ± 50.92	189.00	266.80 ± 43.30	251.00	0.0701 [k]
Hemoglobin	13.46 ± 1.38	13.70	13.02 ± 1.552	12.95	13.30 ± 0.79	13.00	0.7773 [k]
Ferritin	235.0 ± 214.5	169.5	302.1 ± 405.7	133.0	71.33 ± 37.87	83.0	0.3616 [k]
Chitoriosidase	24.53 ± 34.92	9.5	67.35 ± 38.19	71.00	53.30 ± 27.67	62.00	**0.0484** [k]

[k] Kruskal Wallis test

Comparison of GD patient groups according to treatment adherence

There was no significant difference ($p > 0.05$) between the study groups that received regular treatment and those that did not receive regular treatment in terms of patients' age, gender characteristics, GD type, and splenectomy (Table-4).

Lyso-Gb1 in the group receiving regular treatment was significantly ($p < 0.05$) lower than the group not receiving regular treatment. WBC, hemoglobin, and ferritin did not show any significant difference ($p > 0.05$) between the groups receiving regular treatment and those not receiving regular treatment. PLT in the group receiving regular treatment was significantly ($p < 0.05$) higher than the group not receiving treatment. In the regular treated group, chitotriosidase was significantly ($p < 0.05$) lower (Table-4).

When the data obtained were compared according to genotypes, a significant difference was found between the groups only in chitotriosidase levels (Table-5). While chitotriosidase enzyme activity in genotype 1 was found to be significantly lower than that in genotype 2, no significant difference was found between genotypes A and C and genotypes B and C.

Comparison of Laboratory Test Levels Between Regular Treated T1GD and T3GD Patients

The Mann-Whitney U test outcomes offer a compelling narrative about how Type 1 and Type 3 Gaucher Disease patients differ, especially in terms of diagnostic and treatment timelines. Notably, the age at which patients are diagnosed is markedly different, as evidenced by a significant p-value of. 001. This significant variation extends to the initiation of treatment, with an equally significant p-value of. 001, suggesting a divergence in the onset of therapeutic intervention between the two disease types. Doses for both initial and subsequent therapies also diverge between patient groups, indicated by p-values below the. 05 significance level, although these differences are not as pronounced as those observed in age-related factors. In contrast, for Ferritin and Hemoglobin levels, and for White Blood Cells and Chitotriosidase levels, the analysis does not reveal any notable differences, with p-values not meeting the threshold for statistical significance. However, the Platelet Count stands at the cusp of significance with a p-value of. 044, hinting at a potentially noteworthy difference in platelet counts that may require additional scrutiny. Lastly, the p-value of. 569 for Lyso-Gb1 levels points to no significant difference in this biomarker among the two groups of GD patients (Table-6).

In the group receiving regular treatment, WBC, PLT, hemoglobin, ferritin, and chitotriosidase did not change significantly ($p > 0.05$) 6 months after treatment compared to first values, while Lyso Gb1 levels were found to be significantly lower ($p = 0.0074$) (Table-7).

Table-6. Comparison of laboratory test levels between regular treated T1GD and T3GD patients.

Disease type	**Gaucher Type I**				**Gaucher Type III**				**p**	
Variable	**Mean±sd**			**Median**	**Mean±sd**			**Median**		
Lyso-Gb1	67.4	±	62.8	50.12	46.72	±	35.45	47.57	0.600	[m]
WBC	6522	±	2278	5990	7340	±	3578	6590	0.603	[m]
PLT (x10³)	212.2	±	60.6	222.0	266.8	±	43.9	251.0	0.064	[m]
Hemoglobin	12.9	±	1.5	12.6	13.3	±	0.8	13.0	0.415	[m]
Ferritin	256.0	±	352.9	110.0	71.3	±	37.9	83.0	0.315	[m]
Chitoriosidase	48.4	±	41.5	44.5	53.3	±	27.7	62.0	0.634	[m]

[m] Mann-whitney u test

Table-7. Comparison of laboratory levels 6 months later in groups receiving regular treatment.

Variable	First Lyso-Gb1				Second Lyso-Gb1				p	
	Mean±sd			Median	Mean±sd			Median		
Lyso-Gb1	58.22	±	61.42	39.25	45.51	±	43.38	27.17	**0.0074**	w
WBC	6728	±	2547	6500	6966	±	2475	6520	0.585	w
PLT (x10³)	239.6	±	48.6	240.0	237.2	±	49.7	238.0	0.985	w
Hemoglobin	12.8	±	1.4	12.7	12.9	±	1.4	12.9	0.192	w
Ferritin	161.5	±	201.9	77.0	150.9	±	184.2	72.0	0.415	w
Chitoriosidase	47.8	±	35.4	51.0	47.1	±	37.4	50.0	0.583	w

Demographic and Clinical Features of Native Patients

Table-8 presents data on patients diagnosed with GD (T1GD) that received no treatment (G4, native patients). The list includes criteria such as age at diagnosis, age at treatment commencement, genotypic information, surgical history, and lyso-gb1 levels, a biomarker for GD.

The first patient was diagnosed at the age of 2 months but has not yet started treatment. Their genotype shows a combination of N370S and R463H alleles. They have not undergone splenectomy, and their lyso-gb1 level is recorded at 375.92 ng/mL.

The second patient was diagnosed at 19 years of age and began treatment at the same age. Their genotype is N370S/R159M. Similar to the first patient, they have not been splenectomized, and their lyso-gb1 level is considerably higher, at 574.00 ng/mL.

The third patient received their diagnosis at 62 years old, which is also when they started treatment. Their genotype is a homozygous N370S mutation. They have not undergone a splenectomy, and their lyso-gb1 level is 82.01 ng/mL, which is markedly lower than the levels observed in the younger patients.

All the data pertains to individuals with T1GD and none have had a splenectomy. The data also indicates variability in the age of diagnosis, treatment initiation, and lyso-gb1 levels among the patients. However, there is a considerable range in both age at diagnosis and Lyso-gb1 levels, with the youngest having not yet started treatment. The mutations vary across the patients, potentially correlating with the differences in Lyso-Gb1 levels.

Table-8. Demographic and clinical features of native patients.

Variable	**Patient-1**	**Patient-2**	**Patient-3**
Gender	Male	Female	Female
Disease Type	T1GD	T1GD	T1GD
Age at diagnosis	2 months	19 years	62 years
Ferritin Level	81	201	1624
Hemoglobin Level	12.5	11.5	11.3
WBC Count	8090	4570	4370
PLT Count (Nx10³)	170	63	131
Chitoriosidase Level	121	104	53
Lyso-Gb1 Level	375.92	574.00	82.01
Allel I	N370S	N370S	N370S
Allel II	R463H	R159M	N370S
SI	NS	NS	NS

GD: Gaucher Disease; WBC: White blood cells; PLT: Platelet; SI: Surgical Intervention.

DISCUSSION

In this study, we investigated whether Lyso-Gb1 has a role only in the diagnosis of GD or also has a role in disease monitoring that reflects treatment consistency. Our results show that in GD, Lyso-Gb1 is a reliable biomarker for diagnosis and treatment monitoring.

Our first remarkable finding is that the Lyso-Gb 1 level shows a correlation to treatment adherence. In the patient group compatible with treatment chitotriosidase and Lyso-Gb1 were found to be significantly lower than the group that did not comply with treatment. While there was no significant change in the WBC, PLT, hemoglobin, ferritin, and chitotriosidase levels in the patient group receiving treatment in our cohort, which was measured for the second time after 6 months, we found a significant change in only Lyso-Gb1 levels. Our second finding is Lyso-Gb1 levels appear to correlate according to splenectomy supported by our data from the comparison of laboratory test levels according to splenectomy.

Previous studies have found an association between Lyso-Gb1 levels and treatment adherence. While treatment interruptions have been known from case reports and a small case series (9-12) Cozma et al showed that Lyso-Gb1 increased in the group that had 'treatment holidays'. Continuing treatment with SRT may be an option in this patient group whose non-compliance with ERT. While ERT is a treatment method that can only be applied in hospitals under the supervision of a physician in our country, SRT is a per-oral treatment option. Dinur et al proved the important role of Lyso-Gb1 levels in the treatment decisions of patients with GD (13).

We presented in detail the laboratory and clinical features of three native patients from our study cohort. They have a considerable range in both age at diagnosis and Lyso-Gb1 levels. Although Lyso-Gb1 is not included among the criteria for starting treatment for GD within the legal rules in our country, it is legally among the criteria for starting treatment in Israel, which is the country where GD is most common (13, 14). We also believe that Lyso-Gb1 should be among the treatment initiation criteria based on the results we obtained from our study.

Genotype-phenotype correlation is well well-recognized in GD (3, 15). In our cohort, numerically higher plasma Lyso-Gb1 concentrations were observed in patients with non-N370S genotype; no statistically significant difference in Lyso-Gb1 concentration was observed between patients with different disease types or mutation types. Although there is a publication in the literature stating that Lyso-Gb1 level is related to genotype (16), there are also publications showing that there is no significant statistical relationship (17-19). In our cohort of limited size, genotype did not seem to be correlated with the disease course. Further studies that monitor more frequently and in large groups are needed to provide more clear information.

In line with most previous studies, Lyso-Gb1 levels were also correlated with splenectomy in our study population (7, 20). Tylki-Szymanska et al. Reported an interesting study data obtained from 64 GD. Lyso-Gb1 was not dependent on splenectomy status (19). It would be worth a further biochemical investigation of whether the levels of Lyso-Gb1 correlated non-correlated in splenectomized patients in prospective studies.

We have some limitations as follows. First, we did not have pre-treatment Lyso-Gb1 concentrations in the treated cohort. Second, the number of patients in our cohort was a small group of total GD patients in the world. Third, we did not have more measurements in a long follow-up period.

CONCLUSION

In summary, our study shows that Lyso-Gb1 is a promising marker in diagnosis and in evaluating the treatment periods. Since its association with the pathogenesis of the disease, Lyso-Gb1 must be considered when developing new strategies for treatment.

Conflict of interest: No conflict of interest was declared by the authors.

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