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Serum progranulin levels in paediatric patients with Gaucher disease; relation to disease severity and liver stiffness by transient elastography

Azza A. Tantawy[1] | Amira A. Adly[1] | Eman A. Ismail[2] | Nouran Y. Salah[1] | Shereen Abdel Alem[3] | Hesham I. Elsantiel[1]

[1]Pediatrics Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[2]Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[3]Department of Endemic medicine and Hepatology, Faculty of medicine, Cairo University, Cairo, Egypt

Correspondence
Amira A. Adly, 6 A ElSheshini street, Shoubra, Soudia buildings, Cairo, Egypt.
Email: amiradiabetes@yahoo.com

Abstract

Background: Non-invasive screening for liver fibrosis using transient elastography (TE) could be of value in the management of Gaucher disease (GD). Progranulin (PGRN) is a novel disease modifier in GD and an independent marker of liver fibrosis.

Objectives: We determined PGRN levels in paediatric patients with GD and assessed its role as a potential marker for disease severity and relation to liver stiffness by TE.

Methods: Fifty-one GD patients (20 had type 1 and 31 had type 3) with a median age of 9.5 years were compared to 40 age- and sex-matched healthy controls and were studied focusing on visceral manifestations, neurological disease, haematological profile and PGRN levels as well as abdominal ultrasound and TE. Patients were on enzyme replacement therapy (ERT) for various durations and those with viral hepatitis infection were excluded.

Results: By TE, 14 GD patients (27.5%) had elevated liver stiffness ≥7.0 kPa. Liver stiffness was significantly higher in type 1 GD patients than type 3 ($P = .002$), in splenectomized patients ($P = .012$) and those with dysphagia ($P < .001$). Liver stiffness was positively correlated with age of onset of ERT ($P < .001$). PGRN levels were significantly lower in GD patients compared with controls ($P < .001$). PGRN was significantly lower in GD patients with squint ($P = .025$), dysphagia ($P = .036$) and elevated liver stiffness ($P = .015$). PGRN was positively correlated with white blood cell count ($r = .455$, $P = .002$) and haemoglobin ($r = .546$, $P < .001$), while negatively correlated with severity score index ($r = -.529$, $P < .001$), liver volume ($r = -.298$, $P = .034$) and liver stiffness ($r = -.652$, $P < .001$).

Conclusions: Serum PGRN levels were associated with clinical disease severity and elevated liver stiffness in paediatric GD patients.

KEYWORDS

Gaucher disease, liver stiffness, progranulin, severity score index, transient elastography

1 | INTRODUCTION

Gaucher disease (GD), a common lysosomal storage disease, is caused by mutations in *GBA1* with resultant defective glucocerebrosidase function and the consequent accumulation of its substrate, glucosylceramide, in macrophages and other cell types.[1] There are three types of GD based on its neurological complications (type 1 is non-neuropathic, type 2 is acute neuropathic and type 3 is chronic neuropathic). Extra-neurologic systematic features include hepatosplenomegaly, pancytopenia and osteoporosis as a consequence of Gaucher cell infiltration in target organs. Clinical manifestations may have huge variations among patients carrying the same *GBA1* mutations, ranging from very early disease onset to very mild clinical presentations.[2,3] Therefore, it has been speculated that additional disease modifiers exist in GD patients.[4]

Onset in childhood is usually predictive of a severe, rapidly progressive phenotype and children with type 1 GD are at high risk for morbid complications.[5] Complications include chronic liver disease and hepatopulmonary syndrome which has unfavourable outcome.[6] Long-term liver complications that have been associated with GD include fibrosis and cirrhosis.[7]

Evaluating and managing liver disease in patients with GD may be challenging.[8] Screening for liver fibrosis could be of additional value in the management of GD patients.[9] Radiologic methods for staging hepatic fibrosis are emerging as promising tools.[10] One of those methods is FibroScan, or transient elastography (TE), which non-invasively assesses liver fibrosis and presents comparable performance to liver biopsy to predict liver-related outcomes.[11-13] TE can be used to evaluate liver stiffness and hepatic fat deposition in adult patients with cirrhosis, chronic hepatitis and liver transplantation.[14] The advantages of TE include non-invasiveness, a highly successful rate and the reduced requirement of sedation in children.[15]

Although interest and research in the field of GD are rapidly expanding, yet, significant barriers remain in the ability to predict phenotype, assess disease progression and determine optimal treatment strategy on an individual basis.[16] Search for suitable biomarkers to facilitate earlier treatment, monitor progression of disease, as well as the efficacy of therapeutic intervention is mandatory.[16,17]

Progranulin (PGRN), also known as granulin-epithelin precursor (GEP), is recognized for its role in a variety of physiologic and disease processes, including immunomodulation,[18] cell growth, wound healing,[19] host defense [20] and inflammation.[21,22] PGRN acts as an anti-inflammation molecule by direct binding to tumour necrosis factor (TNF) receptors.[22,23] PGRN also functions as an important neurotrophic factor, and mutations of the *GRN* gene (coding PGRN) are directly linked to frontotemporal [24,25] as well as considered contributory to other neurological diseases.[26,27]

PGRN has been shown to play an important role in lysosomes, and homozygous mutation of the *GRN* gene results in neuronal ceroid lipofuscinosis.[28,29] PGRN also functions as a co-chaperone with the heat shock protein 70 (HSP70) disaggregation system. During stress, PGRN-HSP70 prevents the aggregation of lysosomal glucocerebrosidase and lysosomal integral membrane protein 2 (LIMP 2) in the cytoplasm, and facilitates their trafficking to the lysosome. These findings have implications in GD, and PGRN has been reported as a novel disease modifier in GD.[4,30]

Key points

Progranulin levels are low in patients with Gaucher disease and associated with clinical disease severity and elevated liver stiffness. Early start of enzyme replacement therapy may decrease the risk for development of hepatic fibrosis later in life.

Serum PGRN has been found an independent marker of liver fibrosis in patients with biopsy-proven non-alcoholic fatty liver disease (NAFLD).[31] However, to our knowledge, the relation between PGRN and liver fibrosis among patients with GD has not been previously explored. The aim of the study was to determine PGRN levels in paediatric patients with GD and assess its role as a potential marker for disease severity and relation to liver stiffness by TE.

2 | MATERIALS AND METHODS

This cross-sectional study included 51 patients with GD diagnosed on the basis of clinical signs, deficient β-glucosidase enzyme activity in peripheral leucocytes and/or molecular genetic analysis [32-34] and recruited from the regular attendants of the Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University. They included 20 patients with type 1 GD and 31 patients with type 3 GD as classified according to the criteria described.[35,36] The clinical manifestations of the patients were classified using the modified severity scoring index (SSI), which is based on an assessment of the extent of liver, spleen and bone involvement and the severity of pancytopenia.[37] None of the patients received either glucocorticoids and/or chemotherapy or other immunomodulating drugs. None of the studied patients were on anticoagulant therapy at the time of study. All patients were under regular enzyme replacement therapy (ERT) using recombinant glucocerebrosidase (Cerezyme, imiglucerase injection, Genzyme, Cambridge, MA, USA) in a dose of 60 U/kg per 2 weeks.

Exclusion criteria were metabolic syndrome (type 2 diabetes, hypertension or ischaemic heart disease) and known non-Gaucher liver disease (viral hepatitis, human immune deficiency virus, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, Wilson's disease, alpha-1-antitrypsin deficiency and abetalipoproteinaemia).

Another group of 40 age- and sex-matched healthy subjects with no history of inborn errors of metabolism in their first- and second-degree relatives was enrolled as a control group. An informed consent was obtained from each patient or control or their legal guardians before enrolment in the study. This study was approved by the local ethical committee of Ain Shams University and is in accordance with the Helsinki Declaration of 2008.

Patients with GD were subjected to full medical history focusing on haematological manifestations (pallor, bleeding and history of blood transfusion), splenomegaly or splenectomy, hepatic manifestations, neurological symptoms (squint and ophthalmoplegia, convulsions, pseudobulbar manifestations including dysphagia and trismus and manifestations suggestive of cerebellar affection) and history of splenectomy. The size of liver and spleen was assessed and full neurological examination was performed including cranial nerves, motor and sensory systems.

2.1 | Radiological assessment

Abdominal ultrasonography was done to assess the volume of liver and spleen in cubic centimetre (calculated as multiples of normal sizes predicted for body weight).[38]

TE as a non-invasive method replaced the need for liver biopsy in our studied paediatric patients. Liver stiffness measurements were performed using FibroScan® (ECHOSENSE, FIBROSCAN 502, Paris, France). The procedures were performed by an investigator who was blind to clinical and laboratory data. Measurements of liver stiffness were performed on the right lobe of the liver through intercostal spaces in correspondence to the mid-axillary line, while patients have been lying in the supine position with the right arm in maximal abduction. All patients underwent the examination with M probe. Ten valid measurements were performed, and FibroScan® software calculated the median value of liver stiffness expressed in kilopascals (kPa) as reported by Sandrin et al.[11] Only examinations with success rate >60% and interquartile range (IQR; the difference between 75th and 25th percentiles) < 30% of the median were included in this study and were considered reliable. The cut-off of 7.0 kPa was used to identify the presence of significantly elevated liver stiffness.[9,13,39,40]

2.2 | Laboratory analysis

Peripheral blood samples were collected on ethylenediaminetetraacetic acid (EDTA) (1.2 mg/mL) for complete blood count (CBC). For chemical analysis and assessment of PGRN levels, clotted samples were obtained and serum was separated by centrifugation for 15 minutes at 1000 × g then stored at −80°C till subsequent use in enzyme-linked immunosorbent assay (ELISA). Laboratory analysis included CBC using Sysmex XT-1800i (Sysmex, Kobe, Japan) with examination of Leishman-stained smears for red blood cell (RBC) morphology and differential white blood cell (WBC) count, bone marrow aspiration and examination and liver function tests using Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Initial β-glucosidase and chitotriosidase levels as well as molecular analysis of the *GBA* gene were obtained from medical records of patients. Serum ferritin was performed on Cobas e 411 (Roche Diagnostics, Mannheim, Germany). Lyso-GL1 was analysed by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[41] Serum PGRN levels were assessed by ELISA using kit supplied by Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd (Shanghai, China). The assay sensitivity is 5.12 ng/mL and the intra- and inter-assay coefficients of variation are 6.2%-7.6% and 8.1%-9.3% respectively.

2.3 | Statistical analysis

Analysis of data was done using Statistical Package for Social Science (IBM SPSS) version 21 (IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov test was used to examine the normality of data. Quantitative variables were described in the form of range, mean and standard deviation or median and interquartile range (IQR; 75th and 25th percentiles). Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student's *t* test was applied. For comparison of non-parametric quantitative variables between two groups, Mann-Whitney test was used. Qualitative variables were compared using Chi-square (X^2) test or Fischer's exact test when frequencies were below 5. Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, a Spearman correlation test was performed. Multivariable regression analysis was employed to assess the relation between PGRN or liver stiffness and other studied variables. A P value < .05 was considered significant in all analyses.

3 | RESULTS

The clinical, laboratory and radiological data of GD patients are listed in Table 1. GD patients included 29 males and 22 females (ratio, 1.4:1). The median age of GD patients was 9.5 (IQR, 4-14) years. Twenty-one patients had type 1 and 31 had type 3. None of the studied patients was obese or had metabolic syndrome. The control group consisted of 22 males and 18 females (ratio, 1.2:1) and their median age was 10.8 (IQR, 5-14.9) years.

The most common *GBA* genotype among the studied GD patients was L483P (C1448T > C) being found in 38 (74.5%) patients; 33 (64.7%) patients had homozygous C1448T > C (6 had type 1 GD and 27 had type 2 GD) while five patients had heterozygous C1448T > C. Non-L483P genotype was found in 13 (25.5%) patients; four (7.8%) patients had homozygous C1226A > G genotype, three (5.9%) patients had C475C > T; 1226A > G mutations and two (3.9%) patients had homozygous C1342G > C *GBA* genotype while each of following four genotypes was found in only one patient: homozygous C1193G > A, homozygous C754T > C, homozygous C259C > T and C475C > T; 1604 G > A.

3.1 | Liver stiffness by TE among the studied patients with GD

By TE, the mean liver stiffness among GD patients was 6.76 ± 1.9 kPa and 14 (27.5%) GD patients had elevated liver stiffness ≥7.0 kPa (Table 1). As shown in Table 2, liver stiffness was significantly higher

TABLE 1 Clinical, laboratory and radiological data of the studied patients with Gaucher disease

Variable	All patients (n = 51)	Type 1 GD (n = 20)	Type 3 GD (n = 31)
Males, n (%)	29 (56.9)	9 (45.0)	20 (64.5)
Age (years), median (IQR)	9.5 (4-14)	6.5 (3.5-13.5)	9 (5-15)
Age of onset of ERT (years), mean ± SD	3.8 (1.0-6.1)	4.1 (2.8-5.8)	1.5 (1.0-2.9)
Duration of ERT (years), mean ± SD	3.5 (2-11)	2 (1.5-3.5)	8 (2-12)
Positive family history, n (%)	16 (31.4)	5 (25.0)	11 (35.5)
Initial β-glucosidase (umol/L/h), mean ± SD	0.57 ± 0.33	0.65 ± 0.37	0.51 ± 0.29
Initial chitotriosidase (nmol/mL/h), median (IQR)	4229.5 (1560-7900)	3070.5 (1210-8116.5)	5000 (2390-7800)
Genotyping			
L483P (C1448T > C), n (%)	38 (74.5)	10 (50.0)	28 (90.3)
Non-L483P, n (%)	13 (25.5)	10 (50.0)	3 (9.7)
Clinical, Laboratory and radiological data at evaluation			
Weight for age SDS, median (IQR)	-0.66 (−1.9-0.7)	0.03 (−1.02-1.01)	-1.2 (−2.49-0.33)
Height for age SDS, median (IQR)	-1.54 (−2.92 – −0.33)	-1.13 (−2.08 – −0.13)	-1.83 (−3.04 – −0.9)
BMI SDS, median (IQR)	0.64 (−0.81-1.55)	1.11 (0.39-1.71)	0.29 (−1.84-1.46)
Severity Scoring Index, mean ± SD	14 (7-30)	7.5 (4.5-10)	29 (14-32)
Splenectomized, n (%)	6 (11.8)	5 (25.0)	1 (3.2)
Neurological findings			
Squint, n (%)	15 (29.4)	0 (0)	15 (48.3)
Convulsions, n (%)	15 (29.4)	0 (0)	15 (48.4)
Dysphagia, n (%)	12 (23.5)	0 (0)	12 (38.7)
Developmental delay, n (%)	25 (49.0)	2 (10.0)	23 (74.2)
WBC count (x 10^9/L)	7.9 ± 2.8	8.4 ± 3.1	6.5 ± 2.5
Haemoglobin (g/dL), mean ± SD	11.3 ± 1.5	11.3 ± 1.2	11.2 ± 1.7
Platelets (x 10^9/L), mean ± SD	244.7 ± 75.6	276.25 ± 84.9	224.32 ± 71.3
ALT (IU/L), mean ± SD	37.5 ± 6.8	38.1 ± 7.4	35.2 ± 6.1
AST (IU/L), mean ± SD	34.3 ± 8.8	36.1 ± 9.3	32.4 ± 8.4
Lyso-GL1 (ng/mL), median (IQR)	120.5 (83.7-173.3)	97 (49.6-244.7)	131 (92-173)
Serum ferritin (μg/L)	392 (220- 540)	372 (202- 510)	410 (210- 550)
Serum Progranulin (ng/mL), median (IQR)	170 (100-280)	180 (88-328)	150 (100-290)
Liver volume[a], mean ± SD	1.28 ± 0.46	1.28 ± 0.37	1.27 ± 0.52
Spleen volume[a], mean ± SD	1.28 ± 0.38	1.21 ± 0.34	1.32 ± 0.40
Liver stiffness (Kpa)	6.76 ± 1.9	7.74 ± 1.81	6.11 ± 1.58
Liver stiffness			
<7.0 kPa, n (%)	37 (72.5)	12 (60.0)	25 (80.6)
≥7.0 kPa, n (%)	14 (27.5)	8 (40.0)	6 (19.4)

Abbreviations:

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; ERT: enzyme replacement therapy; GD: Gaucher disease; IQR: interquartile range; SDS: standard deviation score; SSI: severity score index.

[a]volume is calculated in multiple of normal for weight.

in type 1 GD patients than type 3 ($P = .002$) and in splenectomized patients ($P = .012$) as well as those with dysphagia ($P < .001$). Liver stiffness was positively correlated with liver volume ($r = .652$, $P < .001$) and age of onset of ERT ($r = .598$, $P < .001$) (Table 3). No correlation was found between liver stiffness and ERT duration. Upon comparison of each group of GD patients separately, the same results were obtained. These variables remain significant in multivariable regression analysis (Table 4).

3.2 | PGRN levels among the studied patients with GD and healthy controls

PGRN levels in GD patients were significantly lower compared with healthy controls (median [IQR], 170 [100-280] ng/mL vs 305 [190-650] ng/mL; $P < .001$). PGRN was significantly lower in GD patients with squint ($P = .025$), dysphagia ($P = .036$) and elevated liver stiffness ($P = .015$) than those without (Table 2). As shown in Table 3,

TABLE 2 Progranulin and liver stiffness in relation to clinical characteristics of the studied patients with Gaucher disease

	Progranulin		Liver stiffness	
Variable	**Median (IQR)**	***P*-value**	**Mean ± SD**	***P*-value**
Sex				
Male	180 (70-300)	.841	6.82 ± 1.59	.274
Female	180 (125-325)		7.13 ± 1.74	
Family history				
Positive	190 (75-280)	.792	6.91 ± 1.05	.362
Negative	175 (123-313)		6.22 ± 1.47	
GD type				
Type 1	180 (88-328)	.314	7.74 ± 1.81	.002
Type 3	150 (100-290)		6.11 ± 1.58	
Genotyping				
L483P (C1448T > C)	185 (125-290)	.063	6.38 ± 1.27	.132
Non-L483P	125 (25-225)		7.18 ± 1.17	
Splenectomy				
Positive	180 (100-320)	.871	7.63 ± 1.51	.012
Negative	203 (170-250)		6.21 ± 1.04	
Squint[a]				
Positive	165 (73-270)	.025	7.01 ± 1.15	.302
Negative	235 (175-350)		6.83 ± 1.01	
Convulsions[a]				
Positive	175 (70-275)	.320	7.48 ± 1.98	.181
Negative	203 (100-338)		6.82 ± 1.75	
Dysphagia[a]				
Positive	150 (50-280)	.036	8.51 ± 2.34	<.001
Negative	220 (120-375)		6.60 ± 1.76	
Developmental delay				
Positive	175 (100-250)	.604	6.51 ± 1.57	.583
Negative	225 (120-300)		7.19 ± 1.76	
Liver stiffness				
<7.0 kPa, n (%)	250 (58-325)	.015	—	—
≥7.0 kPa, n (%)	170 (100-285)			

[a]These complications are only present in patients with type 3 GD. Data were expressed as mean and SD, where comparisons were made using Student's *t* test, or as median (IQR), where Mann-Whitney test was used for comparisons.

PGRN was positively correlated with WBC count and haemoglobin while it was negatively correlated with severity score index, liver volume and liver stiffness (Figure 1). Severity score index, haemoglobin and liver stiffness were the significant independent variables related to increased PGRN levels by multivariable regression analysis (Table 5).

4 | DISCUSSION

Although cirrhosis and portal hypertension have been regarded as rare complications in GD, there is increased risk for liver fibrosis, cirrhosis and hepatocellular carcinoma.[42,43] The exact pathophysiology of liver fibrosis in GD remains unraveled. Hepatic infiltration by Gaucher cells might establish a fibrogenic microenvironment because of the chronic low-grade inflammation.[43] In addition, immunological abnormalities, such as T-cell dysfunction or chronic stimulation of the immune system, have been implicated in the pathophysiology of liver fibrosis in GD patients. Recently, Regenboog et al [44] reported that GD itself could be the explanation for a distorted iron metabolism; however, the co-existence of pathological mutations in the *HFE* gene should not be missed. *HFE* status may act as a genetic risk factor for iron storage in patients with GD.[45]

It has been reported that in GD, a chronic low-grade inflammation state can lead to high ferritin levels and increased hepcidin transcription with subsequent trapping of ferritin in macrophages.[46]

TABLE 3 Correlation between progranulin and liver stiffness and the studied clinical, laboratory and radiological variables in patients with Gaucher disease

	Progranulin		Liver stiffness	
	r	*P*-value	*r*	*P*-value
Age (years)	.131	.494	−.065	.734
BMI SDS	.208	.143	.170	.370
Age of onset of ERT (years)	−.224	.114	.598	<.001
Duration of ERT (years)	−.102	.475	.114	.304
Severity score index	−.529	<.001	.292	.118
Initial β-glucosidase (umol/L/h)	.178	.211	−.183	.334
Initial Chitotriosidase (nmol/mL/h)	.171	.230	−.113	.551
WBC count (x 10^9/L)	.455	.002	.240	.201
Haemoglobin (g/dL)	.546	<.001	−.236	.209
Platelets (x 10^9/L)	.151	.291	.037	.844
ALT (IU/L), mean ± SD	−.138	.417	−.218	.324
AST (IU/L), mean ± SD	−.246	.371	−.169	.268
Lyso-GL1 (ng/mL)	.106	.461	.018	.925
Serum ferritin (μg/L)	.213	.315	.161	.294
Progranulin (ng/mL)	–	–	−.652	<.001
Liver volume[a]	−.298	.034	.652	<.001
Spleen volume[a]	−.002	.991	.070	.728
Liver stiffness (Kpa)	−.652	<.001	—	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ERT, enzyme replacement therapy; SDS, standard deviation score; WBC, White blood cell.

[a]volume is calculated in multiple of normal for weight.

TABLE 4 Multivariable linear regression analysis of the relation between liver stiffness and the studied variables in patients with Gaucher disease

	Unstandardized Coefficients		Standardized Coefficients	
	B	Standard Error	Beta	*P* value
(Constant)	2.955	0.530		<.001
Age of onset of ERT (years)	0.254	0.024	0.324	.043
Liver volume[a]	0.007	0.002	0.495	.003
Progranulin (ng/mL)	−0.003	0.001	−0.359	.019

Abbreviation: ERT, enzyme replacement therapy.

[a]volume is calculated in multiple of normal for weight.

Recently, Lefebvre et al [47] explored the iron status of a large cohort of 90 type 1 GD patients, including 66 patients treated with ERT. Serum levels of hepcidin remained within the physiological range, while the transferrin saturation was slightly decreased in children. Inflammation-independent hyperferritinaemia was found in 65% of the patients. Treated patients exhibited reduced hyperferritinaemia, increased transferrin saturation and transiently increased systemic hepcidin.

Better understanding of liver involvement in GD can spare patients from unnecessary invasive testing, and assist physicians in decision-making when evaluating patients with GD suspected for significant liver disease.[8] Numerous studies have demonstrated the ability of TE to predict liver fibrosis, cirrhosis and cirrhosis-related complications in various chronic liver diseases. TE and magnetic resonance elastography (MRE) techniques have been used to identify and monitor GD patients with regard to the presence of liver fibrosis.[9,13,40,48] An unknown association between PGRN and GD has been found and PGRN has been identified as an essential factor for glucocerebrosidase's lysosomal localization.[4] The role of PGRN in liver disease and fibrosis has been shown in some studies but not in GD.[31,49]

By TE, 14 (27.5%) GD patients had elevated liver stiffness ≥7.0 kPa. Liver stiffness was significantly higher in type 1 GD patients, in association with splenectomy and dysphagia. Liver stiffness was positively correlated with age of onset of ERT. This is may be related to the delay of diagnosis of those patients, delay in age of onset of ERT (because of the poor disease awareness and difficult access to treatment) and high incidence of splenectomy among those patients.

In agreement with our results, Bohte et al [9] performed a pilot study that included 14 type 1 GD patients (seven splenectomized and seven non-splenectomized) and seven healthy controls. Their study was the first to demonstrate that liver stiffness values, measured by TE and MRE, were significantly higher in splenectomized GD patients when compared with non-splenectomized GD patients. TE measurements correlated significantly with MRE and with chitotriosidase activity. However, TE was successful and identified high stiffness of the liver in two splenectomized GD patients where MRE failed because of the high liver iron load.[9] It has been reported that splenectomy may be an indication of a more severe GD and partial splenectomy is sometimes recommended to preclude excessive uptake of infused enzyme by the reticuloendothelial splenic tissue.[37] The differences with regard to liver stiffness between splenectomized and non-splenectomized GD patients and the observation that splenectomy can be avoided by ERT suggest that ERT may decrease the risk for development of hepatic fibrosis and cirrhosis-related complications in the future.[9] Our results further support starting ERT at an early age to avoid the occurrence of liver fibrosis later in life.

Moreover, Webb et al [49] addressed whether elastography technique can serve as a tool for evaluating patients with GD. Their study included 42 patients with type 1 GD and 33 patients with cirrhosis as well as 22 healthy volunteers. Liver stiffness in GD as measured by TE and shear wave elastography (SWE) was slightly higher than in the healthy controls, but much smaller than for the cirrhotic patients. They concluded that TE and SWE show a significant promise as non-invasive and reproducible tools to differentiate GD from healthy controls and cirrhotic patients.

Nascimbeni et al [40] used TE to assess the prevalence of significant liver fibrosis in 37 adult type 1 GD patients and identify its

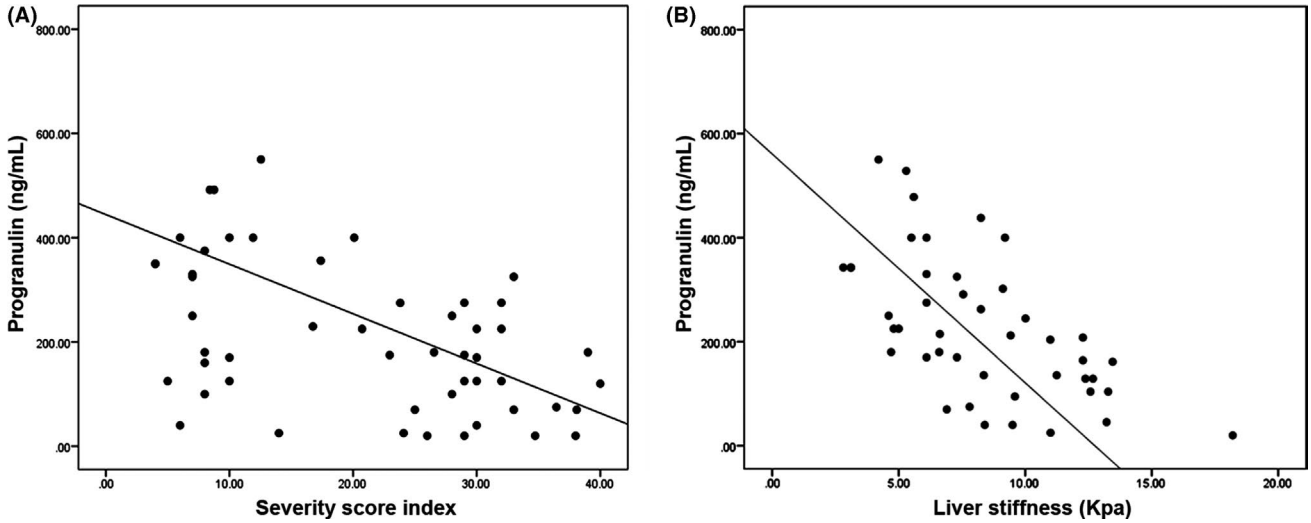

FIGURE 1 Correlation between progranulin and severity score index (A) and liver stiffness (B)

TABLE 5 Multivariable linear regression analysis of the relation between progranulin and the studied variables in patients with Gaucher disease

	Unstandardized Coefficients		Standardized Coefficients	
	B	Standard Error	Beta	*P* value
(Constant)	−320.750	246.808		.206
Severity score index	−3.572	0.373	−0.590	<.001
Haemoglobin (g/dL)	50.114	14.443	0.477	.002
WBC count (x 10^9/L)	30.629	23.092	0.185	.197
Liver volume[a]	0.032	0.139	0.038	.823
Liver stiffness (Kpa)	−44.738	13.669	−0.543	<.001

Abbreviations: ERT, enzyme replacement therapy; WBC, White blood cell.

[a]volume is calculated in multiple of normal for weight.

predictors among GD-related variables, ERT and metabolic features. The median liver stiffness was 4.6 [3-15.1] kPa and seven patients (19%) had significant fibrosis, which was associated with splenectomy, severity scores and biomarkers (ACE and HDL-cholesterol) of GD severity. In GD patients on stable ERT, GD severity, non-N370S *GBA1* genotypes, diastolic blood pressure, BMI and the number of metabolic syndrome components emerged were major GD-related predictors of liver fibrosis. The length of ERT was inversely correlated with liver disease in GD patients, suggesting a beneficial effect of ERT on liver fibrosis.

Recently, Lipiński et al [13] evaluated clinical utility and relevance of TE by FibroScan in 59 Polish patients (55 adults and 4 children) with GD (43 patients with type 1 and 16 patients with type 3) aged 7-86 years. They assessed two parameters, controlled attenuation parameter (CAP) and liver stiffness, in regard of GD-related variables, type of GD, age of patients, ERT and metabolic features. Elevated CAP was present in 23% of type 1 GD patients and 19% of type 3 GD patients. Elevated liver stiffness was present in 21% of type 1 GD patients and 13% of type 3 GD patients. Liver stiffness was positively correlated with the age of start of ERT. They concluded that TE by FibroScan could be performed in GD patients with increased BMI and especially those with metabolic syndrome as they have other important risks for liver disease.

Liver TE is a non-invasive procedure that can evaluate liver stiffness and monitor treatment efficacy in paediatric liver disease.[15] Chin et al [15] evaluated the therapeutic effect of liver stiffness in a children with GD that was monitored by liver elastography after ERT. They demonstrated through TE that ERT may reverse the liver elastic texture within 3 months of therapy in a paediatric patient with GD. Further studies are warranted to assess whether the changes are universal to paediatric patients with GD, especially if the changes could guide the treatment for late-treated patients with GD.

Although our patients were on variable duration of ERT, yet, elevated liver stiffness by TE was evident in some patients despite normal liver functions. It has been reported that elevated serum transaminases are present in 30% to 50% of patients with GD before treatment begins.[50] The accumulation process of glucosylceramide (GL1) and its deacylated lysolipid, glucosylsphingosine (lyso-GL1) usually does not affect liver function and therefore, serum transaminases tend to be within the normal range for most GD patients and severe abnormalities are more likely to be seen in splenectomized patients.[8,51]

In our study, PGRN levels in GD patients were significantly lower compared with healthy controls. In line with our results, Jian et al [4] measured serum levels of PGRN in 115 GD patients and 99 healthy controls. They found that serum PGRN levels were significantly lower in GD patients than those in healthy controls of the general population and of Ashkenazi Jews. The authors also identified four *GRN* gene single nucleotide polymorphisms (SNPs), including rs4792937,

rs78403836, rs850713 and rs5848, and three point mutations in a full-length *GRN* gene sequencing in 40 GD patients. Large-scale SNP genotyping in 161 GD and 142 healthy controls was conducted and the four SNP sites have significantly higher frequency in GD patients. Moreover, PGRN null mice develop GD-like phenotypes, including typical Gaucher-like cells in lung, spleen and bone marrow. Recombinant PGRN was found to be therapeutic in various animal models of GD and human fibroblasts from GD patients. These findings not only provide new insight into the pathogenesis of GD, but may also have implications for diagnosis and alternative targeted therapies for GD.[4]

Interestingly, PGRN was significantly lower in GD patients with squint and dysphagia. Positive correlations were observed between PGRN and WBC count as well as haemoglobin, while PGRN was negatively correlated with severity score index. This indicates that low PGRN was associated with a more severe GD phenotype and PGRN could be used for early identification of disease progression. Moreover, PGRN was significantly lower in GD patients with elevated liver stiffness and negatively correlated with liver volume and liver stiffness. Liver stiffness was one of the significant independent variables related to increased PGRN levels by multivariable regression analysis.

To the best of our knowledge, no previous studies assessed PGRN in relation to disease severity or liver stiffness among GD patients. After validation of our results in further prospective studies, we suggest that measurement of serum PGRN levels may allow early detection of liver disease among GD patients before reaching fibrosis stage. PGRN has been shown to be linked with chronic liver injury and fibrosis progression in both humans and experimental models.[52,53] The role of PGRN as an independent marker of liver fibrosis has been shown in patients with biopsy-proven NAFLD. Yilmaz et al [31] found that serum PGRN levels were significantly higher in NAFLD patients than in controls and were associated with the degree of hepatic fibrosis but not related to the degree of steatosis or necroinflammation. With regard to the mechanisms linking altered levels of PGRN with hepatic fibrogenesis, Yilmaz et al [31] speculated that this can occur via the regulation of the hepatocyte growth factor/mesenchymal-epithelial transition factor system signalling pathway.

Recently, Yoo et al [49] explored the role of PGRN in liver injury in two different models of chronic liver disease: methionine-choline-deficient diet (MCD)-induced NASH and carbon tetrachloride (CCl4)-induced liver fibrosis. In the CCl4-induced fibrosis model, PGRN showed protective effects against hepatic injury, inflammation and fibrosis via inhibition of nuclear transcription factor kappa B (NF-κB) phosphorylation. PGRN also decreased lipid accumulation and inhibited pro-inflammatory cytokine production and fibrosis in the MCD-induced NASH model. Furthermore, PGRN suppressed inflammatory and fibrotic gene expression in a cell culture model of hepatocyte injury and primary stellate cell activation. These findings suggest PGRN delivery as a potential therapeutic strategy in chronic inflammatory liver disease.

Although our study was a cross-sectional one and we could not compare PGRN levels before and after ERT, Afinogenova et al [54] evaluated serum levels of PGRN, cathepsins D and S as well as YKL-40 in GD patients compared with established GD biomarkers, chitotriosidase, chemokine ligand 18 (CCL18) and other indicators of disease severity and response to therapy. The mean PGRN levels were lower in GD patients compared with healthy controls. No change was found in PGRN levels after ERT. PGRN was not correlated with chitotriosidase or CCL18. The authors concluded that these biomarkers do not meet robust properties of established macrophage-specific biomarkers but they may inform severity of skeletal disease, contribution of fibrosis to residual splenomegaly and other disease manifestations. These findings, including markedly low PGRN levels that do not change upon ERT, are intriguing to prompt further investigations to decipher their role in pathophysiology and relevance to diverse phenotypes of GD.[54]

One limitation of our study is that its cross-sectional nature which could not imply causality and the relatively small sample size. The lack of gene analysis for *GRN* gene in the studied GD patients is also an important limitation. Therefore, further larger longitudinal studies are needed to identify the role of PGRN in predicting liver fibrosis among paediatric patients with GD.

In conclusion, elevated liver stiffness by TE is evident among paediatric patients with GD. Early start of ERT may decrease the risk for development of hepatic fibrosis later in life as shown by the positive correlation between liver stiffness and age of onset of ERT. Splenectomy may be an indication of a more severe GD. PGRN levels are low in GD patients and associated with clinical disease severity and elevated liver stiffness. Further larger longitudinal studies before and after the start of ERT and gene analysis for *GRN* gene in patients with GD are needed to verify the practical utility of PGRN measurement and role as a biomarker of liver fibrosis in GD.

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CONFLICT OF INTEREST

Nothing to declare. The manuscript has not been submitted elsewhere nor previously published. The corresponding author, on behalf of all authors, hereby states that all authors have contributed to the manuscript in significant ways, have reviewed and agreed upon the manuscript content.

ORCID

Amira A. Adly https://orcid.org/0000-0003-2309-9771
Eman A. Ismail https://orcid.org/0000-0002-1162-0171
Nouran Y. Salah https://orcid.org/0000-0001-6507-8835
Shereen Abdel Alem https://orcid.org/0000-0002-3612-0130

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