Pompe Disease Screening in a Sample of Iranian Patients

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Abstract- The reason why the experiments were done: previous studies have shown that the incidence rates vary in different populations. Now we report the preliminary results of the screening study. Pompe disease is a rare but potentially treatable disorder caused by the deficiency of the lysosomal enzyme acid-α-glucosidase (GAA). GAA activity was measured on DBS in 65 patients with undiagnosed myopathies presenting to the hospital of the research setting in Isfahan, Iran, from 2016 to 2017 and then was confirmed by genetic analysis. Of the total of 65 patients, 29 (44.6%) were male, and 36 (55.4%) were female. The mean age of the patients was 29±12.55 years, and their mean age at the disease onset was 17±12.75. Two patients (one male and one female) were diagnosed with a low acid alpha-glucosidase activity. Only one patient (female) showed a compound heterozygotic mutation of the GAA gene (c.-32-13T>G). Early diagnosis of Pompe disease is important for improving the outcome.

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Keywords: Pompe disease; Lysosomal enzyme; Proximal myopathy; HyperCKemia

Introduction

Pompe Disease (PD; GSD II) is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme acid-α-glucosidase (GAA) and leading to the generalized accumulation of lysosomal glycogen, especially in the heart, skeletal and smooth muscles as well as the nervous system (1). A deficiency of this enzyme results in neuromuscular symptoms, ranging from severe early-onset disease, which presents with hypertrophic cardiomyopathy, muscular hypotonia, hepatomegaly, and cardio-respiratory failure (1-3) to juvenile and Later-Onset PD (LOPD), which has milder disease progression that can range from isolated asymptomatic hyperCKemia (4) to slowly progressive muscle weakness (5) and has a diaphragm involvement in up to 20% of the cases (5).

The diagnosis of LOPD is a challenging endeavor. Muscle biopsy is used as a diagnostic tool for detecting metabolic myopathies such as Pompe disease (6), but in 9-30% of the cases, a muscle biopsy is nonspecific or normal in LOPD patients (7-10). The rarity of the disorder, histopathological variability, the broad phenotypic spectrum, and variable diagnostic approaches in different countries lead to delays or failure in the diagnosis of LOPD (11,12).

Effective enzyme replacement therapy has been available since 2006. ERT was less effective in patients with LOPD than in infants (12-14), and since earlier diagnosis is important for prognosis, the development of more rapid diagnostic techniques such as the Dried Blood Spot (DBS) to detect GAA activity can contribute to an earlier LOPD diagnosis (15,16).

The present study thus examined patients with undiagnosed myopathy or proximal myopathies in the lower limbs or those with more proximal rather than distal involvement in terms of the prevalence of Pompe disease using the DBS as the main screening tool.

Materials and Methods

Research design and sampling

A total of 65 patients with undiagnosed myopathies presenting to the hospital of the research setting in Isfahan, Iran, were investigated from 2016 to 2017. The research project was approved by the ethics committee of the Isfahan University of Medical Sciences. Written consent was obtained from each participant before

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enrollment in the study. All the patients were enrolled in the study based on the inclusion criteria.

Inclusion and exclusion criteria

We performed a DBS of patients with suspected LOPD: (1) Age ≥1 year; and (2) Proximal myopathies in the lower limbs or symptoms of Limb-Girdle Muscle Weakness (LGMW) or proximal and distal muscle involvement (proximal >distal) of an unknown etiology.

All the patients underwent an electromyography examination, and seven of the 65 underwent a muscle biopsy examination as well. They were then followed by genetic testing when required.

Data collection

The DBS samples were sent to the metabolic laboratory, Hamburg University Medical Center that specialized in metabolic disorders, so as to measure their GAA activity using fluorometry techniques. GAA activity in the DBS was assessed using fluorometry with the methylumbelliferyl-α-D-glucoside substrate (17). Based on the filter paper containing Acarbose, the depressed activity of Alpha-1, 4-glucosidase was deemed pathological if found to be <0.9 nmol/spot 21h (18). Genetic, molecular analysis was performed [Centogene AG (Rostock, Germany), ARCHIMED Life Science GmbH (Vienna, Austria)] for all patients with reduced enzyme activity. For genetic analysis, the GAA gene was analyzed by Sanger sequencing.

Statistical analysis

The statistical analysis of the data was carried out in SPSS-25.0. The results are presented as mean (±SD).

Results

Of the total of 65 patients, 29 (44.6%) were male, and 36 (55.4%) were female. The mean age of the patients was 29±12.55 years, and the mean age at the disease onset was 17±12.75 years. A total of 40 patients (61%) presented with hyperCKemia (CK >170 U/l), 34 (52%) with LGMW, 12 (18%) with proximal myopathy of the lower limbs, and 19 patients (29%) with proximal and distal muscle involvement. Of the recruited patients, 41 (63%) had a positive family history of myopathy, and 41 (63%) were children of consanguineous marriages. Six of the recruited patients (9.2%) presented with facial paresis, two (3.1%) with cardiomyopathy, and 17 patients (26.2%) with skeletal deformities. Table 1 summarizes the clinical features and laboratory data of all the patients.

In the present study, two patients (one male and one female) were diagnosed with low acid alpha-glucosidase activity. Only one patient (one female) (1.53%) received positive genetic confirmation of Pompe disease.

Table 1. The results of the clinical and neurological examination

Clinical findings (N=65)		Mean±SD
Age (year)		29.50±12.55
Age at onset		17.46±12.75
	Male	29(44.6)
	Female	36(55.4)
	Being parents	41(63.1)
	Family history	41(63.1)
	HyperCKemia	40 (61)
	LGMW	34(52)
Gender frequency (percentage)	Proximal myopathy of lower limb	12(18)
	Proximal and distal involvement	19(29)
	Facial paresis	6(9.2)
	Dysphagia	2(3.1)
	Respiratory Distress	3(4.6)
	Cardiomyopathy	2(3.1)
	Skeletal deformities	17(26.2)

Limb Girdle Muscle Weakness: LGMW; HyperCKemia: CK >170 U/l

Case

The patient was a 35-year-old female. Her chief complaint was muscular exertion intolerance associated with fatigue and muscle cramps, and also LGMW. The symptoms had begun at the age of 25. There was no family history of neuromuscular disease involved. The CK value was 471 U/l. The GAA activity was 0.31 nmol/spot 21 h. The electromyography revealed a myopathic pattern in the proximal muscles with myotonic discharge. She had not undergone any muscle biopsies. The human genetic examination for Pompe disease showed a compound heterozygotic mutation of the GAA gene (c.-32-13T>G). No signs of respiratory problems or cardiomyopathy were found in the patient.

Discussion

Pompe disease is a rare disorder with an estimated worldwide incidence of 1/40000 (19). Nonetheless, the exact prevalence is not known because the disease can be difficult to diagnose, and there may be a larger number of undiagnosed patients. Data from the Pompe Registry (20) indicate that 20% of patients have Infantile-Onset Pompe Disease (IOPD). Several studies have shown that the incidence rates may vary in different populations from 1 in 14,000 to 1 in 30,000 (21). Taiwanese populations had a higher incidence of Pompe disease, with the rate of IOPD being 1/57000 and LOPD 1/261500 (22), and the incidence was 1/14000 in African-American populations (19). Musumeci *et al*., evaluated 1051 individuals, and 30 (2.9%) were diagnosed with DBS in the initial screening (23). From a sample of 3076 individuals with LGMW and hyperCKemia, 232 individuals (7.6%) had low GAA activity on the DBS (24).

In one study, the prevalence of Pompe disease was reported as 3.22% in a high-risk population in Tehran (25); in the present study, which was conducted in Isfahan, this rate was 1.53%; the lower incidence in the present study may be due to the smaller number of patients who were enrolled in the study and also because patients with isolated hyperCKemia were not included in this study.

Pompe disease can present anytime, ranging from right after birth through late adulthood, and there is no definitive genotype-phenotype correlation for it (26); however, an earlier onset of symptoms is associated with more severe disease and a greater chance of cardio-respiratory failure. In LOPD, the initial symptoms are usually related to proximal muscle weakness and also respiratory failure (5). In the present study, the patients presented with exercise intolerance, cramps, and LGMW without respiratory distress.

Diagnosis has often been made by muscle biopsy in LOPD (27,28), however, there is a significant chance for false-negative results due to the sporadic accumulation of glycogen in the muscle fiber or washed-out glycogen during processing. Our case had not undergone any muscle biopsies.

Another test was to measure GAA activity in the blood, which has been proven problematic in the past for obtaining a pure lymphocyte sample; however, the direct measurement of GAA enzyme activity using the DBS has recently emerged as a valid screening method (27-29) that is fast and safe and has minimal invasiveness (28); however, in some other screening trials, it has led to false-positive results, perhaps due to incorrect storage or long transports (23,26,28,30). One case of a false positive result was also observed in the present study.

In this group of patients, the DBS assay was used as the main screening tool to check GAA activity, and one sample was found with low GAA activity on the DBS.

The most common mutation found in Caucasians is c.-32-13T>G (also known as IVS1). This mutation was found in more than 70% of children and adults (31), and this case also showed this mutation.

The entire (100%) Iranian population showed hyperCKemia, 95.7% showed LGMW, and 4.3% showed proximal myopathy of the lower limbs (25), and the case reported also presented with exercise intolerance and LGMW.

Since the introduction of ERT (Myozyme and Lumizyme), there has been an increased awareness about this disease, and patients with LGMW should be screened for it using DBS.

Beyond this, we selected patients from the hospital of the research setting in Isfahan, Iran, with genetically unclassified LGMD and limb-girdle muscle weakness and then screened for Pompe disease by enzyme analysis on dried blood spots. Hereby, we report the preliminary results of the screening study.

References

1. van der Ploeg AT, Reuser AJ. Pompe's disease. Lancet 2008;372:1342-53.
2. Engel AG, Seybold M, Lambert E, Gomez M. Acid maltase deficiency: comparison of infantile, childhood, and adult types. Neurology 1970;20:382.
3. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, et al. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. Orphanet J Rare Dis 2012;7:88.
4. Hagemans M, Winkel L, Van Doorn P, Hop W, Loonen M, Reuser A, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. Brain 2005;128:671-7.
5. Echaniz Laguna A, Carlier RY, Laloui K, Carlier P, Salort-Campana E, Pouget J, et al. Should patients with asymptomatic pompe disease be treated? A nationwide study in France. Muscle Nerve 2015;51:884-9.
6. Stenzel W, Schoser B. Inherited and acquired muscle weakness: a moving target for diagnostic muscle biopsy. Neuropediatrics 2017;48:226-32.
7. Laforet P, Nicolino M, Eymard B, Puech J, Caillaud C, Poenaru L, et al. Juvenile and adult-onset acid maltase deficiency in France: genotype–phenotype correlation. Neurology 2000;55:1122-8.

8. Feeney EJ, Austin S, Chien YH, Mandel H, Schoser B, Prater S, et al. The value of muscle biopsies in Pompe disease: identifying lipofuscin inclusions in juvenile-and adult-onset patients. Acta Neuropathol Commun 2014;2:2.
9. Genge A, Campbell N. Reevaluating muscle biopsies in the diagnosis of Pompe disease: a Corroborative report. Can J Neurol Sci 2016;43:561-6.
10. Golsari A, Nasimzadah A, Thomalla G, Keller S, Gerloff C, Magnus T. Prevalence of adult Pompe disease in patients with proximal myopathic syndrome and undiagnosed muscle biopsy. Neuromuscul Disord 2018;28:257-61.
11. Müller-Felber W, Horvath R, Gempel K, Podskarbi T, Shin Y, Pongratz D, et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. Neuromuscul Disord 2007;17:698-706.
12. Toscano A, Montagnese F, Musumeci O. Early is better? A new algorithm for early diagnosis in late onset Pompe disease (LOPD). Acta Myol 2013;32:78-81.
13. Chien YH, Hwu WL, Lee NC. Pompe disease: early diagnosis and early treatment make a difference. Pediatr Neonatol 2013;54:219-27.
14. Ravaglia S, Pichiecchio A, Ponzio M, Danesino C, Garaghani KS, Poloni GU, et al. Changes in skeletal muscle qualities during enzyme replacement therapy in late-onset type II glycogenosis: temporal and spatial pattern of mass vs. strength response. J Inherit Metab Dis 2010;33:737-45.
15. Kallwass H, Carr C, Gerrein J, Titlow M, Pomponio R, Bali D, et al. Rapid diagnosis of late-onset Pompe disease by fluorometric assay of α-glucosidase activities in dried blood spots. Mol Genet Metab 2007;90:449-52.
16. La Marca G, Casetta B, Malvagia S, Guerrini R, Zammarchi E. New strategy for the screening of lysosomal storage disorders: the use of the online trapping-and-cleanup liquid chromatography/mass spectrometry. Anal Chem 2009;81:6113-21.
17. Spada M, Porta F, Vercelli L, Pagliardini V, Chiadò-Piat L, Boffi P, et al. Screening for later-onset Pompe's disease in patients with paucisymptomatic hyperCKemia. Mol Genet Metab 2013;109:171-3.
18. Lukacs Z, Nieves Cobos P, Mengel E, Hartung R, Beck M, Deschauer M, et al. Diagnostic efficacy of the fluorometric determination of enzyme activity for Pompe disease from dried blood specimens compared with lymphocytes-possibility for newborn screening. J Inherit Metab Dis 2010;33:43-50.
19. Valle DL, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, Hirschhorn R, et al. The metabolic and molecular bases of inherited disease. In: Glycogen storage disease type II. Acid alpha-glucosidase (acid maltase) deficiency. USA: McGraw-Hill, 2001:3389-420.
20. Byrne BJ, Kishnani PS, Case LE, Merlini L, Müller-Felber W, Prasad S, et al. Pompe disease: design, methodology, and early findings from the Pompe Registry. Mol Genet Metab 2011;103:1-11.
21. Ausems M, Verbiest J, Hermans M, Kroos M, Beemer F, Wokke J, et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. Eur J Hum Genet 1999;7:713-6.
22. Chien Y-H, Lee N-C, Huang H-J, Thurberg BL, Tsai F-J, Hwu W-L. Later-onset Pompe disease: early detection and early treatment initiation enabled by newborn screening. J Pediatr 2011;158:1023-7. e1.
23. Musumeci O, la Marca G, Spada M, Mondello S, Danesino C, Comi GP, et al. LOPED study: looking for an early diagnosis in a late-onset Pompe disease high-risk population. J Neurol Neurosurg Psychiatry 2016;87:5-11.
24. Lukacs Z, Cobos PN, Wenninger S, Willis TA, Guglieri M, Roberts M, et al. Prevalence of Pompe disease in 3,076 patients with hyperCKemia and limb-girdle muscular weakness. Neurology 2016;87:295-8.
25. Tehrani KHN, Sakhaeyan E, Sakhaeyan E. Evaluation prevalence of Pompe disease in Iranian patients with myopathies of unknown etiology. Electron Physician 2017;9:4886-9.
26. Amartino H, Painceira D, Pomponio R, Niizawa G, Sabio Paz V, Blanco M, et al. Two clinical forms of glycogen-storage disease type II in two generations of the same family. Clin Genet 2006;69:187-8.
27. Neuromuscular AAo, Medicine E. Diagnostic criteria for late-onset (childhood and adult) Pompe disease. Muscle Nerve 2009;40:149-60.
28. Vissing J, Lukacs Z, Straub V. Diagnosis of Pompe disease: muscle biopsy vs blood-based assays. JAMA Neurol 2013;70:923-7.
29. Kishnani PS, Steiner RD, Bali D, Berger K, Byrne BJ, Case LE, et al. Pompe disease diagnosis and management guideline. Genet Med 2006;8:267-88.
30. Goldstein JL, Young SP, Changela M, Dickerson GH, Zhang H, Dai J, et al. Screening for Pompe disease using a rapid dried blood spot method: experience of a clinical diagnostic laboratory. Muscle Nerve 2009;40:32-6.
31. Van Capelle CI. Children with Pompe disease: clinical characteristics, peculiar features and effects of enzyme replacement therapy [dissertation]. Erasmus University Rotterdam., 2014.