

Evaluation prevalence of Pompe disease in Iranian patients with myopathies of unknown etiologyKhadijeh Haji Naghi Tehrani¹, Elmira Sakhaeyan², Elnaz Sakhaeyan³

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Type of article: Original

Abstract

Background: Pompe disease is a rare but potentially treatable metabolic disorder having an estimated worldwide incidence of one in forty thousand live births. While the introduction of enzyme replacement therapy (ERT) has considerably increased the awareness of the disease, the delay in diagnosis is still consistent and most patients go undetected.

Objective: This study aimed to determine the prevalence of late-onset Pompe disease (LOPD) in a high-risk population, using dried blood spot (DBS) as a main screening tool.

Methods: This cross-sectional study was performed on the 93 patients who attended to the neuromuscular center of Bu-ali hospital in Tehran, Iran, during 2014-2015. Inclusion criteria were: 1) age ≥ 1 years, 2) proximal myopathies of unknown etiology in lower limbs or symptoms of limb girdle muscle weakness (LGMW), and 3) unexplained elevated CPK (>174). Acid α -glucosidase (GAA) activity was measured separately on DBS by fluorometric method. For the final diagnosis, GAA deficiency was confirmed by a biochemical assay in skeletal muscle, whereas genotype was assessed by GAA molecular analysis. All statistical tests were performed using the SPSS version 16. Results are presented as mean (SD) or median (IQR), as appropriate.

Results: In a 12-month period, we studied 93 cases: 5 positive samples (5.3%) were detected by DBS screening, biochemical and molecular genetic studies finally confirmed LOPD diagnosis in 3 cases (3.22%). Among the 93 patients, 100% showed hyperCKemia, 89 patients (95.7%) showed LGMW and 4 patients had symptoms of proximal myopathies in the lower limb.

Conclusions: Results from the LOPED study suggest that GAA activity requires accurate screening by DBS in all patients referred for hyperCKemia and/or LGMW.

Keywords: Pompe disease, Proximal myopathy, hyperCKemia

1. Introduction

Pompe disease, also called glycogen storage disease type II, (GSD II) is a rare autosomal recessive disorder due to acid α -glucosidase (GAA) deficiency leading to glycogen accumulation in several tissues, with predilection of skeletal muscle and heart (1). Clinically, it is associated with a range of phenotypes, with variable organ involvement, age of onset and severity degree (2). Late-onset Pompe disease (LOPD) is a slowly progressive form with juvenile or adult presentation, characterized by progressive muscle weakness and often, respiratory impairment (3). Diagnosis of LOPD is still challenging and often quite delayed. This has been hypothesized to be due to several reasons such as rarity of the disorder, wide clinical spectrum, overlap of signs and symptoms with other neuromuscular disorders, or variable diagnostic approach in different countries (4). Recently, Kishnani et al., (5) using data from the International Pompe Registry, calculated the time interval between onset and diagnosis, 'a diagnostic gap', in different categories of patients with Pompe disease, and still found this delay consistent. First carried out in 2006, enzyme replacement therapy (ERT) represents the first disease-specific treatment. In patients

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Received: May 24, 2016, Accepted: September 26, 2016, Published: July 2017

iThenticate screening: September 29, 2016, English editing: May 12, 2017, Quality control: June 16, 2017

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with LOPD, ERT was less effective in older juveniles and adults than in infants, likely because of delayed diagnosis (4, 6). These considerations suggested development of more rapid diagnostic tools, such as the dried blood spot (DBS), to detect GAA activity, which could result in an earlier LOPD diagnosis (7-10). To the best of our knowledge, there are study that estimated the prevalence of LOPD among the Iranian population. This study was aimed to determine the prevalence of late-onset Pompe disease (LOPD) in a high-risk population, using the dried blood spot (DBS) as a main screening tool.

2. Material and Methods

2.1. Research design and setting

This cross-sectional study was performed on patients with suspected neuromuscular disorders who attended the neuromuscular center of Bu-ali hospital from 1 January 2014 to December 2015, in Tehran, Iran. The study was approved by the ethical committee of Islamic Azad University, Tehran Medical Sciences Branch, and written informed consent for participation was obtained.

2.2. Sampling

Regarding the study type, all the patients who attended the neuromuscular center of Bu-ali hospital were consecutively enrolled into the study, based on the inclusion criteria.

2.3. Inclusion and exclusion criteria

Inclusion criteria were 1) age \geq year old, 2) proximal myopathies of unknown etiology in lower limbs or symptoms of limb girdle muscle weakness (LGMW), and 3) unexplained elevated CPK (CPK $>$ 174). Exclusion criteria were: 1) age $<$ 1 year old, and 2) myopathies with known or explained etiology. Blood samples were collected, immediately spotted on filter paper (DBS), and dried at room temperature.

2.4. Data collection

DBS samples were sent to laboratories specialized in metabolic disorders, to be examined by experts in measuring GAA activity, by fluorometry techniques. For each patient, clinical history and laboratory data were collected in a Case Report Form (CRF). GAA activity in DBS was assessed by a fluorometry method using the substrate 4-methylumbelliferyl- α -D-glucoside (11). The interval between the 99th centile activity of the disease range (5.78 nM/h/mL) and the first centile activity of the normal controls (6.10 nM/h/mL) was considered as the cut-of target range, and the cut-off for α -glucosidase activity at 6 nM/h/mL on DBS. Patients who were considered positive were those with GAA activity below 6 nM/h/mL.

2.5. Statistical analysis

All statistical tests were performed using the SPSS software package Version 16 (SPSS Inc, Chicago, Illinois, USA). Results are presented as mean (SD) or median (IQR), as appropriate. The patients, recruited from one Iranian neuromuscular center, were all admitted for diagnostic purposes.

3. Results

Of a total of 93 patients, 42 patients (45.2%) were male, and 51 patients (54.8%) were female. The mean age of the patients was 27 ± 11.02 years. All the patients presented with hyperCKemia, 95.7% (89 patients) with LGMW and 4.3% (4 Patients) with proximal myopathy of the lower limbs. Of the recruited patients, 33 (35.5%) had a positive family history of myopathy. Forty (43%) of the recruited patients were children of consanguinity. In our study, five patients (three female and two male) were diagnosed with low level of acid alpha glucosidase activity. Only three out of five patients (one female and two male) received a positive genetic result as confirmation of Pompe disease. Hence, the Pompe disease prevalence was reported as 3.22%. Using the fluorometric method, reduced GAA enzyme activity in DBS was found in five participants (5.3%). A confirmatory biochemical test performed on skeletal muscles of the five patients resulted positive at the DBS testing; GAA deficiency was confirmed in 3/5 patients (3.22%). Among the three patients with LOPD, 1/3 was female (33.33%); the mean age at disease onset was 17.66 years, while the median age at disease diagnosis was 30.66 years. All three of these patients had hyperCKemia and proximal myopathy of the lower limbs.

4. Discussion

Pompe disease is a rare disorder having an estimated worldwide incidence of 1 in 40 000 live births. However, several studies have suggested that incidence rates may vary among different ethnic populations with a fluctuating range from 1 in 14 000 to 1 in 300 000 (12). Since the introduction of ERT, either among neuromuscular experts or

general practitioners, the awareness of this disease has considerably increased; but, unfortunately, the delay of diagnosis is still consistent, especially in LOPD (5), because of the rarity of the disorder and the variable clinical presentation or overlapping of symptoms with other neuromuscular disorders. The recent studies have tried to improve the approach to a rapid and correct diagnosis using the DBS method in targeted populations. The DBS GAA enzyme assay test is a robust, rapid and reliable first tier test for screening Pompe disease, thus it is recommended to be performed for patients with unexplained myopathies suspected of Pompe disease

- 1) Genetic confirmatory tests are recommended to confirm diagnosis of Pompe disease
- 2) Increased awareness of Pompe disease, clinical signs and symptoms and diagnostic algorithm is needed to help more timely diagnosis and to prevent irreversible sequelae
- 3) With due attention to prevalence rate in our study, assessment of patients with symptoms of limb girdle myopathy /proximal myopathy plus unexplained elevated CPK -especially when orthopnea is present, is recommended

As shown in several studies, early diagnosis is of high importance in LOPD patients. The earlier the treatment is started, the better the outcome will be. In Taiwan, in 2005, a nationwide NBS program for Pompe disease was initiated with highly successful results. So far, a large number of newborns have been screened for Pompe disease (over 400 000 infants). Of those screened, six infants were found to carry the infantile-onset Pompe disease (LOPD-prevalence 1/57 000), and 20 cases of LOPD and 294 cases of pseudo deficiency were also detected (13, 14). On the other hand, considered as key features, specific signs or symptoms, usually represented by hyperCKemia and/or unclassified LGMW, Pompe disease has been screened by DBS, although in a limited number of patients and in different ethnic populations. Recently, Spada et al. (11) screened 137 patients with unclassified hyperCKemia and found a 2.2% prevalence of Pompe disease. A 2013 study by Preisler et al. (15) using the DBS method to evaluate the prevalence of Pompe disease in undetermined patients with limb-girdle muscular dystrophy, identified three patients with Pompe disease in 38 individuals screened (8%). Consequently, in the present study, in a period of 12 months, we were able to collect 93 samples from patients with suspected neuromuscular disorders because of proximal LGMW with hyperCKemia. In this group of patients, we used the DBS assay as the main screening tool to check GAA activity: we found, five samples with low GAA activity on DBS, but after the biochemical confirmatory assay on skeletal muscle, LOPD diagnosis was assessed in three patients (3.22%). Among those patients, 100% showed hyperCKemia, 95.7% showed LGMW, and 4.3% manifested with proximal myopathy of the lower limbs. The prevalence of Pompe disease is reported as 3.22%. A 2006 study by Fernandez et al., (16) retrospectively reviewed muscle biopsy specimens of 104 patients with hyperCKemia and found Pompe disease in four patients (3.8%). Although Vissing et al., (17) in a recent diagnostic review, indicated the 'blood-based' assays as a prevalent diagnostic tool, muscle biopsy still had an important role. The interpretation of DBS results needs great accuracy and experience. In fact, the results may indicate some false positives (9). This also happened in our study, considering that we first obtained five positive samples; finally, three patients were biochemically confirmed with a diagnosis of LOPD. A possible explanation of those slightly controversial results could be due to the quality of samples. It is well known that DBS specimen collection and storage can be crucial for a correct analysis (18).

5. Conclusions

The result of this study revealed that LOPD study suggests that GAA activity should be accurately screened by DBS in all patients referring for hyperCKemia and/or LGMW. However, future multicenter study with larger sample size should be performed to estimate the prevalence the LOPD among the Iranian population.

Acknowledgments:

This study was supported by Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran. The funding organizations had no role in the design or conduct of the study; or the collection, management, or analysis of the data; or the preparation, review, or approval of the manuscript.

Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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