Prevalence of Gaucher disease in patients with unknown cause of splenomegaly and/or thrombocytopenia in Saudi Arabia: a multicenter cross-sectional study protocol

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ABSTRACT

Background: Gaucher disease (GD) is the most common prevalent lysosomal storage disorder characterized by the accumulation of glucosylceramide within the lysosomes of cells that are ordinarily degraded to glucose and lipid components. The primary objective of this study is to determine the prevalence of GD in the high-risk group (i.e., patients with splenomegaly and/or thrombocytopenia of unknown cause).

Methods: This multicenter, cross-sectional study will enroll patients with signs of splenomegaly and/or thrombocytopenia with no definitive cause over 12 months. Eligible patients will be assessed for acid β-glucosidase and acid sphingomyelinase enzymes activity using dried blood spot samples. A total of 400 participants from Saudi Arabia were enrolled in the study.

Results: The analyses of this study were descriptive, and the sample size was chosen to permit the collection of sufficient data in order to determine the prevalence of GD in a high-risk group. Consequently, the sample size was not assessed in terms of statistical power, but rather precision based on the expected frequency.

Conclusion: Saudi Arabia is the largest country in the Arabian Peninsula, with a population of more than 28 million. Despite healthcare being freely accessible to Saudi citizens, several potential barriers to healthcare access and individual healthcare-seeking have been reported. While GD is a rare disease, its incidence in Saudi Arabia appears to be higher than in other parts of the world. Nevertheless, no previous nationwide study was conducted to provide reliable data regarding the incidence and characteristics of Saudi patients with GD. Furthermore, the published literature is scarce regarding the treatment patterns and outcomes of GD in Saudi Arabia, as well. Early diagnosis of GD can potentially reduce disease progression and improve patients’ quality of life. Therefore, our study data will improve the local practice and increase the awareness toward GD and acid sphingomyelinase deficiency in Saudi Arabia.

Keywords: Lysosomal storage disorders, Gaucher disease, acid sphingomyelinase deficiency, dried blood spot, glucocerebrosidase, epidemiology, Saudi Arabia.
Gaucher disease with splenomegaly and/or thrombocytopenia

disease (GD) is the most commonly encountered form of sphingolipidoses with an estimated global rate of 1.5-2.5 per 100,000 live births [4]. The incidence of GD also shows substantial ethnic disparity, with the highest incidence manifested among the Ashkenazi Jewish population with an incidence as high as 1 per 800 live births [5].

The disorder was initially described in the early 1880s in a patient with a massive spleen of unknown etiology; since then, the cumulative body of evidence has identified GD as an autosomal, recessive disease, resulting from mutations in the GBA1 gene, with subsequent disturbance in the lysosomal level/activity of glucocerebrosidase enzyme [6]. Under physiological conditions, glucocerebrosidase (also known as acid β-glucosidase) degrades glucocerebroside.

Patients with GD exhibited excessive accumulation of glucocerebroside within the lysosome and development of Gaucher cells [7]. Subsequently, Gaucher cells, which are characterized by a “crumpled tissue paper” appearance, invade hematological tissues, such as bone marrow and spleen, and other body organs, leading to different phenotypes of GD [8]. Rarely, GD may develop secondary to a deficiency in saposin C, an activator of glucocerebrosidase [9]. Alongside the development of Gaucher cells, it has been elucidated that the pathogenesis of GD involves other metabolic pathways such as sphingosine leading to the premature death of neural cells [10,11].

Clinically, GD is staged into three distinct forms according to the severity of the presentation and neurological involvement. Type 1 GD is the mildest and the most common phenotype of GD, whose presentation can range from asymptomatic/mild constitutional symptoms to splenomegaly, hepatomegaly, gallstones, bone pain and pathological fractures, disturbed mineral density, and thrombocytopenia [12]. Less commonly, patients with type 1 GD may present with interstitial fibrosis, proteinuria, and ocular complications [4].

Type 2 and 3 GD are characterized by additional neurological complications such as ophthalmoplegia, epilepsy, and behavioral changes. They differ from each other regarding the onset of these neurological sequel [13,14]. Defective glucocerebrosidase activity below 10%-15% of the regular activity is the confirmatory test of choice for diagnosing GD [15]. GD is currently managed by enzyme replacement therapy and substrate reduction therapy to reduce the risk of GD-related complications and improve the quality of life of affected patients [16].

On the other hand, acid sphingomyelinase deficiency (ASMD) is another autosomal, recessive disease resulting from acid sphingomyelinase’s defective activity. Patients with ASMD present heterogeneous phenotypes, ranging from asymptomatic disease to infantile fatal neurodegenerative disorder [17]. Nonetheless, splenomegaly, osteopenia, and pulmonary complications represent the most common presentations of ASMD [18]. Previous reports estimated that ASMD affects 1 in every 250,000 individuals. However, because some cases go misdiagnosed or undiagnosed, it is difficult to determine the true frequency of ASMD in the general population [19]. Since there is considerable symptomatic overlap between GD and ASMD, parallel testing with GD has been identified as a strategy for improving the diagnosis of ASMD.

In the Middle East, especially Saudi Arabia, a higher prevalence of inborn error of metabolism was observed compared to reported rates from European countries and the United States [20]. The high rate of consanguinity was postulated as the main contributing factor to this high incidence [21]. However, gaps in the evidence on the epidemiology of GD patients in Saudi Arabia are yet to be addressed.

Rationale

This study aims to determine the prevalence of GD and ASMD in Saudi Arabia by screening patients with unknown causes of splenomegaly and/or thrombocytopenia, in addition to describing the other comorbidities in these patients and reducing the delay in GD and ASMD diagnosis in Saudi Arabia. These data will change the local practice and increase the awareness toward GD and ASMD in Saudi Arabia.

The primary objective of this study is to determine the prevalence of GD in the high-risk group. The high-risk group is defined as patients with splenomegaly and/or thrombocytopenia of unknown cause.

The secondary objectives are to determine the prevalence of ASMD; describe the demographic and clinical characteristics of patients with GD and ASMD; compare these characteristics to those of the rest of the screened population, and describe other comorbid conditions in patients with GD and ASMD.

Methods

This study is a multicenter, cross-sectional study with an interventional diagnostic procedure of dried blood spot (DBS) test and genetic testing. All patients meeting the inclusion criteria in the outpatient setting of 25 collaborating institutions that cover different regions of Saudi Arabia will be enrolled.

All patient data, except for the result of the investigation, will be collected in a single visit.

Each enrolled patient will visit the investigator for a baseline visit. The investigator will contact them later to collect investigation of blood test results. During the baseline visit, data and blood samples for enzyme tests and genotyping will be collected by the investigator/designated person at the site.

Both male and female patients ranging between age 2 to 75 years of Saudi nationality or non-Saudi nationality
will be included in this study. Informed consent will be obtained from the patient/guardian. All patients presenting with clinical, instrumental, or laboratory signs of splenomegaly or thrombocytopenia over a 12-month period will be evaluated. In addition, patients with at least one of these characteristics will be tested for acid β-glucosidase and acid sphingomyelinase enzyme activity on DBS samples and patients with decreased activity of these enzymes will be enrolled.

The splenomegaly will be defined as a palpable spleen at ≥1 cm from the costal margin or diagnosed by ultrasound scans, magnetic resonance imaging, or computed tomography of the spleen. Thrombocytopenia will be defined as <150,000/mm³ and is suspected to be immune thrombocytopenia. Thrombocytopenia with at least one of the following conditions will be required to test for GD or ASMD: history of bone or joint abnormalities such as pain, pathological fractures, arthritis, radiological bone disease, joint stiffness, hemorrhosis, monoclonal gammopathy of unknown significance, polyclonal gammopathy in patients ≤30 years, hemoglobin levels <12 g/dl in men and <11 g/dl in women, and history of splenectomy.

Patients with splenomegaly and/or thrombocytopenia that have been confirmed with a diagnosis including GD/ASMD or any other definitive disorder will be excluded. Moreover, patients with splenomegaly due to portal hypertension, documented by abdomen ultrasound scan or another instrumental test, and hematological malignancies, confirmed by positive results in the physical examination and peripheral blood smear or bone marrow fine-needle aspiration or bone marrow biopsy will also be excluded. Patients with hemolytic anemia and thalassemia, except for sickle cell anemia, will also not be included in the analysis. Subjects who have already undergone DBS testing will be excluded.

The study will be conducted in specialty care centers: focusing on pediatric hematologists, adult hematologists, and hematopathologists. Therefore, hospitals with specialty care centers for adult and pediatric hematology will be targeted, which have substantial experience with patients having splenomegaly with or without thrombocytopenia.

The analyses of this study will be descriptive in nature, and the sample size has been chosen to permit to collect the sufficient data to determine the GD prevalence in high-risk groups. Consequently, the sample size has not been assessed in terms of statistical power, but rather precision based on the expected frequency [95% confidence interval (CI)].

Motta et al. [22] revealed that the prevalence of GD in patients with splenomegaly and/or thrombocytopenia was 3.6% (95% CI = 1.4-7.2; 1/28 patients) diagnosed by acid β-glucosidase enzyme activity on DBS testing. The 95% CI when investigating outcomes of various frequencies depend on sample size and expected prevalence as follows:

\[ CI = p \pm z \times \sqrt{\frac{p(1-p)}{n}} \]

with \( n \) as the number of the target population per country, \( p \) is the estimated proportion (relative precision), and \( z = 1.96 \) for \( a = 5\% \).

Therefore, using the anticipated prevalence of GD in patients with splenomegaly and/or thrombocytopenia of 3.6% will result in a sample size of 400 patients considering the 95% CI [1.8%: 5.4%] with a 5% margin of error, as presented in Table 1.

Table 2 demonstrates the study flow chart and data collected per visit. All patient data, except the result of the investigation, will be collected in a single visit. Each enrolled patient will visit the investigator for a baseline visit. The investigators will complete the electronic case report form (eCRF) soon after the baseline visit. Upon receiving the test results, investigators are required to report in the eCRF (within five days) that the results will be provided to their respective patients.

The following data will be collected from each patient: the sociodemographic profile, findings of physical examination, history of comorbidities, history of previous surgeries, family history of inborn errors of metabolism, presenting symptoms, duration of splenomegaly or thrombocytopenia, findings of complete blood count, erythrocyte sedimentation rate, C-reactive protein, liver function test, renal function test, findings of imaging examinations, electrocardiogram (ECG) findings, echocardiographic findings, ophthalmologic examination, pulmonary function test (spirometry/plethysmography), biomarkers (such as target region

<table>
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<tr>
<th>Table 1. The sample size and corresponding expected prevalence (95% CI).</th>
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<tr>
<td><strong>Expected sample size of patients with splenomegaly and/or thrombocytopenia</strong></td>
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</table>
Gaucher disease with splenomegaly and/or thrombocytopenia

amplification polymorphism, chitotriosidase, CCL18, and lyso-sphingomyelin), and findings of DBS screening. In addition, a central laboratory will provide DBS and genetic tests service to all sites in the study (ARCHIMED Life Science Laboratories, Vienna).

An independent contract research organization (CRO) will provide the study centers with the proper levels of access, grants, and privileges to eCRFs that the investigator or the authorized designee will fill according to the complete guidelines. Data entry screens development, validation rules programming, and study database maintenance will be the independent CRO’s responsibility. The computerized handling of the data by the CRO may generate additional queries automatically identified through preprogrammed and tested validation rules. Validation rules will be detailed in the data validation plan. In addition to automatic validation rules, manual medical review of data may generate further queries that will be raised on the system. Site staff will resolve automatic and manual queries by confirming or modifying the data questioned through the electronic data capture system. Data collection and validation procedures will be detailed in an appropriate operational study manual. The general logistic aspects of the study are shown in Figure 1.

Demographics including age, gender, and ethnicity.

Vital signs including weight, height, heart rate, and blood pressure.

Medical history will include the history of symptoms and signs from the inclusion criteria, recording of all physical manifestations any other medical history.

Laboratory data and medical records will include any available data of radiological imaging, cardiac examination, skeletal examination, neurological examination, ophthalmologic examination, pulmonary function test, biomarkers, hematological/biochemical, liver function test, and surgeries.

e Disease diagnosis including GBA and acid sphingomyelinase enzyme activity in DBS and DNA analysis of GBA gene and acid sphingomyelinase enzyme only as confirmatory for positive DBS.

The study’s primary endpoint will be estimating the prevalence of GD in the high-risk group tested by acid β-glucosidase enzyme activity DBS testing. Other endpoints will include the prevalence of ASMD in the high-risk group, description of demographic profile and patient characteristics, and frequency of comorbid conditions in patients with GD.

All data collected during the study will be analyzed in the appropriate descriptive analyses. Statistical analyses will be carried out by Statistical Package for the Social Sciences version 18 or higher. The prevalence of GD and ASMD will be described using frequency and percentages with a 95% CI. Other variables will be described using mean ± standard deviation for continuous variables and counts for categorical variables. Patients’ variables will

<table>
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<tr>
<th>Evaluation/data point</th>
<th>Study visit</th>
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<tr>
<td>Eligibility: inclusion/exclusion criteria</td>
<td>✓</td>
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<tr>
<td>Informed consent</td>
<td>✓</td>
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<tr>
<td>Demographic profile</td>
<td>✓</td>
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<tr>
<td>Splenomegaly and thrombocytopenia assessment</td>
<td>✓</td>
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<tr>
<td>Physical examination/ vital signs</td>
<td>✓</td>
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<tr>
<td>Medical history</td>
<td>✓</td>
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<tr>
<td>Family history of GD/ASMD or any other genetic disease</td>
<td>✓</td>
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<tr>
<td>Laboratory data and medical record</td>
<td>✓</td>
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<tr>
<td>Disease diagnosis</td>
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<td>Disease symptoms</td>
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<tr>
<td>Concomitant medications form (classes only)</td>
<td>✓</td>
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<tr>
<td>Adverse drug reactions and adverse events related to specific study procedure</td>
<td>✓</td>
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be compared using Mann-Whitney-Wilcoxon tests for continuous parameters and Chi-square for categorical parameters. A probability value \( p \)-value of less than 5% will be considered significant.

**Primary analysis**

The prevalence of GD will be described using frequency and percentages with 95% CI. This analysis will be descriptive and will be conducted on the eligible population, patients in the high-risk group and fulfilling the inclusion criteria, who came to the selected centers during the specified study period and signed an informed consent form.

**Secondary analysis**

The prevalence of ASMD will be described using frequency and percentages with a 95% CI. This analysis will be descriptive and will be conducted on the eligible population, patients in a high-risk group and fulfilling the inclusion criteria, who came to the selected centers during the specified study period and signed an informed consent form.

Baseline demographics including age, gender, risk factors of family history of GD/ASMD, and ethnicity of patients with confirmed GD and patients with confirmed ASMD will be compared to the rest of the population using Mann-Whitney-Wilcoxon tests for continuous parameters and Chi-square for categorical parameters. This analysis will be a comparative analysis conducted between the GD/ASMD disease-positive population and all patients from the eligible population.

Medical history and comorbidities, including lab data and medical records, will be described using frequency (percentages) with 95% CI. This analysis will be descriptive and will be conducted on the GD positive population, all patients from the eligible population for whom GD was diagnosed positive by DBS test and genetic testing.

This section provides specifications for preparing the final statistical analysis plan (SAP), which will be issued before database lock. Therefore, any differences compared to this statistical section should be identified and documented in the final SAP.

**Central laboratories**

A central laboratory will provide DBS and genetic tests service to all sites in the study.

Table 3 summarizes the laboratory testing services available for the study.

### Results

The analyses of this study will be descriptive in nature. The sample size has been chosen to permit the collection of sufficient data to determine the prevalence of GD in the high-risk group.

Our protocol was reviewed and approved by the Institutional Review Board (IRB) of the included centers including King Fahad Medical City, Prince Sultan Military Medical City, Asser Central Hospital, Prince Saud Bin Jalawi Hospital, King Fahad University Hospital, Qatif Central Hospital, and Maternity and Children Hospital. The study is supervised and funded by Sanofi Genzyme, Saudi Arabia. According to our sample size calculation, we expect at least 400 patients from different sites in Saudi Arabia to be enrolled in our study.

**Discussion**

The Arab region represents one of the most affected areas regarding the high incidence of congenital and genetic disorders [23]. High consanguinity rates, which reach up to 60% in some regions, high prevalence of hemoglobinopathies and metabolic disorders, relatively high maternal and parental age, and lack of proper genetic screening were reported as contributing factors for the high prevalence of genetic disorders in the Arab world [23-25].

In Saudi Arabia, the situation appears to be no different. Previous retrospective studies showed a relatively high incidence of genetic diseases such as inborn error of metabolism, including GD and ASDM. Unfortunately, the prevalence of GD in Saudi Arabia is not available in the published literature, and it is expected to be high and remains undiagnosed.

In 2004, a study was conducted to assess common genetics and metabolic diseases in Riyadh, Saudi Arabia, over 10 years. The GD incidence was 6% out of all genetic metabolic disorders. The study also stated that one of the main reasons for autosomal recessive diseases is the heavily consanguineous marriages, accounting for 60-70% of all marriages in Saudi Arabia [26].

However, there are concerns about the generalizability of the published studies from Saudi Arabia on the total population of the Kingdom since data are generated from one region of the center. Therefore, there is a need for multicenter studies to reflect the real epidemiology of GD and ASDM in Saudi Arabia. Another concern is the lack of nationwide newborn screening programs that can help accurately estimate GD incidence in Saudi Arabia.

<table>
<thead>
<tr>
<th>Testing service</th>
<th>Laboratory / Address</th>
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<tr>
<td>DBS and genotyping</td>
<td>ARCHIMED Life Science Laboratories, Vienna</td>
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Table 3. Testing service and their associated centralized laboratory.
Gaucher disease with splenomegaly and/or thrombocytopenia

GD is a chronic, progressive disorder with multisystem affection. Although patients with GD usually present with very distinctive physical and cognitive features, most of the patients with GD are asymptomatic at birth [27]. Early diagnosis of GD can potentially reduce disease progression and improve patients’ quality of life. Therefore, newborn screening methods are promising modalities for optimizing GD outcomes [28].

With the introduction and availability of tandem mass spectrometry methods, it has become feasible to implement newborn screening programs for many metabolic disorders in both developed and developing countries. Recently, lysosomal storage disorders screening programs have gained much attention, and pilot lysosomal storage disorders programs were conducted in several countries [29,30]. These reports demonstrated several feasible, effective, and affordable methods for lysosomal storage disorders screening programs that can be extended to the larger population [28].

Despite these advancements, it is still challenging to implement these screening programs in Saudi Arabia due to multiple local barriers. The main obstacle is the lack of clear data about the incidence rate and the absence of available treatment options to make early diagnosis meaningful. Therefore, there is a need to provide reliable data about implementing newborn screening in Saudi Arabia. We believe that the outcomes of our study will address this imperative need.

Recently, the DBS has evolved as a new, noninvasive, and reliable technique for assessing the enzymatic activity of many lysosomal storage disorders. The DBS technique depends on immunocapture, inhibitors, or acarbose to stop the enzyme activity within the blood sample. Previous reports showed that DBS effectively identified adult patients with lysosomal storage disorders. Recent guidelines and consensuses recommend DBS as an initial screening test as negative test result likely excludes GD diagnosis. If the DBS shows reduced enzymatic activity, confirmatory testing should be performed by measuring enzymatic activity in fibroblasts/muscle tissue cultures or genetic testing [31,32].

Acknowledgment

We would like to thank Dr. Hussien Ahmed, MD and Dr. Ahmed Salah, MD, from RAY-contract research organization for providing medical writing and editorial support for this protocol manuscript.

List of Abbreviations

- ASMD: Acid sphingomyelinase deficiency
- CRO: Contract research organization
- DBS: Dried blood spot
- eCRF: Electronic case report form
- GD: Gaucher disease

Conflict of interest

Aly Ezzat, Marwan El Bagoury, Sherif Roushdy, and Yahia Aktham are employees of Sanofi Genzyme.

Funding

The study is conducted by Sanofi Genzyme Saudi Arabia, Tahlia Street, Nojoud Center, Gate C, Jeddah, Saudi Arabia. Sanofi Genzyme provided investigator fees to the investigators according to the study’s agreement.

Sanofi Genzyme will provide the needed materials for all study steps, including protocol formation, data collection, medical writing, and manuscript submission. Furthermore, in accordance with local and international regulations, Sanofi Genzyme Saudi Arabia will provide all the required resources needed for the study to be conducted and published. Also, we confirm that the study protocol has been peer-reviewed by the representative of Sanofi Genzyme before submission.

Consent to participate

In case of any presentation of case reports, consent for publication will be obtained from that person, or in the case of children, their parent, or legal guardian.

Ethical approval

The study’s protocol was approved by the ethics committee of King Fahd Medical city for participant hospitals: Qatif Central Hospital, Qatif; Asser Central Hospital, Abha; Prince Saud Bin Jalawi Hospital, Hofuf; King Fahd Hospital, Hofuf; and Maternity and Children Hospital, Asser. The study protocol was registered by the IRB of King Abdulaziz City for Science and Technology (KACST). Also, the protocol was registered and approved by the ethics committee of Prince Sultan military medical city (Protocol number: PIR 16352). This study is being conducted in accordance with the principles laid by the 18th World Medical Assembly (Helsinki, 1964) and all subsequent amendment. This study is being conducted in accordance with the European guidelines for Good Epidemiology Practice. Furthermore, all necessary regulatory submissions (e.g., IRB/IEC) are performed according to Saudi Arabia’s local regulations, including local data protection regulations. Before study enrollment, an informed consent form will be taken from each participant or guardian.

Author contributions

All authors contributed equally to idea generation, protocol formation, protocol writing and editing, and approving the final submitted version of the manuscript.

Availability of data and materials

Data sharing does not apply to this article as no data sets were generated or analyzed during the current study.

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