The Frequency of Fabry Disease among Young Cryptogenic Stroke Patients in the City of Sakarya

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Background: Fabry disease (FD) is known as a rare cause of stroke. Recent studies suggested that FD is an underdiagnosed entity among young stroke patients. We aimed to investigate the frequency of FD in young cryptogenic stroke patients who lived in the City of Sakarya and to define the clinical features that help in recognizing patients with FD. *Methods:* Acute ischemic stroke patients aged 18-55 years who were admitted to our hospital between October 2013 and September 2016 were evaluated for inclusion. Patients with other recognized causes of stroke were excluded. The screening was performed for alpha-galactosidase A (α-Gal A) activity on dried blood spot, and DNA was sequenced for *GLA* mutation in patients with low plasma α-Gal A activity. *Results:* Among the 484 acute ischemic stroke patients, 54 (24 male, 44.4%) young cryptogenic stroke patients were enrolled. The α-Gal A activity was detected as low in 3 patients. *c.[680G > A] p.[R227Q]* missense mutation was identified in 2 male patients. The frequency of FD was calculated as 3.7%. *Conclusions:* Our research is the first FD screening study in Turkish stroke patients. Our results underlined the importance of considering FD during the etiologic evaluation of young cryptogenic stroke patients as it is a rare but potentially treatable entity. **Key Words:** Fabry disease—cryptogenic stroke—X-linked—genetic screening.

Introduction

Stroke is one of the leading causes of morbidity and mortality among young adults. However, even after extensive investigations, the etiology of stroke in young adults cannot be determined in 25%-50% of the cases.[1] Recent studies have shown that 1.2% of young stroke patients with unexplained etiology have Fabry disease (FD).[2] Although it is known to be a rare cause of stroke, recent studies have suggested that FD may be an underdiagnosed disease among young stroke population.

FD or Anderson-Fabry disease is a lysosomal storage disease with X-linked recessive inheritance. The disease is known to be associated with *GLA* gene mutations, which are located at Xq22 encoding the alpha-galactosidase A (α-Gal A) enzyme. Mutations of the *GLA* gene result in deficient enzyme activity causing a progressive intralysosomal accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids in various organs. This excessive lysosomal deposition is responsible for the multiorgan involvement of this systemic disease.[3]

FD is clinically divided into 3 groups according to the level of α-Gal A activity. The α-Gal A enzyme activity is lower than 1% in the classical form. The symptoms, including angiokeratomas, acroparesthesia, corneal opacity, hearing deficit, gastrointestinal disorders, and hypohidrosis, are onset in childhood or adolescence. The clinical symptoms that arise with major organ involvement are left ventricular hypertrophy, renal dysfunction, and stroke, which mostly occur within the third to fifth decade and are related to significant morbidity and mortality. The

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variant form is adult onset, and the patients have detectable α-Gal A activity. Cardiac or renal involvement is the predominant feature of the variant phenotype.[3,4] An intermediate form with milder symptoms and residual α-Gal A activity has also been described.[5]

The proposed pathophysiology of the systemic vasculopathy is a progressive accumulation of globotriaosylceramide in the vascular endothelium leading to ischemia and multifocal infarction of small vessels.[3] The presence of a prothrombotic state, abnormalities in cerebral blood flow velocity, autonomic dysfunction, and increased production of reactive oxygen species is thought to play a role in stroke development.[6]

The diagnosis of FD is challenging in patients with uncommon clinical features. However, diagnosing FD in patients presenting with stroke is crucial, as it has come to be considered one of the manageable causes of stroke after enzyme replacement therapy (ERT). ERT is a promising treatment that could prevent unfavorable outcomes and vital organ damage during the course of the disease by reducing the GL-3 levels and clearing the accumulations of lysosomal depositions from the blood, tissue, and vascular endothelial cells. Early diagnosis and treatment would provide more benefit for patients.[7]

Previous studies have shown that the incidence of FD is 1:117,000 in the general population, and ranges from 1:40,000 to 60,000 in males.[3,8] The aim of this study was to evaluate the frequency of FD in the City of Sakarya to determine the impact of disease burden in young patients with cryptogenic stroke.

Materials and Methods

Study Population

Our institute receives all of the young cryptogenic stroke cases in the City of Sakarya as a sole third-level hospital. Patients aged 18-55 years who were admitted to our hospital with acute ischemic stroke between October 2013 and September 2016 were included in this study. Ischemic stroke diagnosis was made by a neurologist clinically and was confirmed by brain computed tomography scan and magnetic resonance imaging (MRI).

Exclusion criteria were defined according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification.[9] The risk factors for cerebrovascular disease, including arterial hypertension, atrial fibrillation, diabetes mellitus, smoking, hyperlipidemia, family history of stroke, previous stroke, and transient ischemic attack (TIA), were obtained from each patient's medical history. Patients with the diagnosis of atrial fibrillation, acute myocardial infarction, valvular heart disease, other cardioembolic sources of stroke, substance abuse, hematologic disease, stenosis >50% in the cerebral vessels, history of carotid endarterectomy, carotid dissection, vasculitis, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), or pregnancy were excluded from the study. Patients' demographic data, smoking habits, detailed medical history, and findings on neurologic examination were all recorded. None of the patients had previously been diagnosed with FD or had a family history of FD.

Analysis of α-Gal A Activity

Measurement of enzyme activity in dried blood spot (DBS) samples was performed via the DBS technique using filter paper containing DBS as a source of DNA.[10] Four drops of the blood were transferred to a filter paper, allowed to dry at room temperature, and stored at 2-4°C until analysis. The enzyme activities were calculated in μmol/L/h. Patients with values <1.2 μmol/L/h were considered to have low α-Gal A activity.

Genetic Analysis of GLA *Mutation*

In patients with low α-Gal A activity, screening of the *GLA* mutation was performed using DBS cards based on Sanger sequence analysis (ARCHIMED Laboratory, Vienna, Austria). This assay uses polymerase chain reaction amplification followed by Sanger DNA sequencing to detect mutations in the *GLA* gene, which is found on *Xq22* and spans 13 kb of genomic DNA (7 exons, cDNA of 1290 bases). The coding sequences and flanking intronic sequences (minimum of 20 base pairs) of exons 1-7 of the *GLA* gene are amplified from purified genomic DNA and sequenced in the forward and reverse directions. Sequencing of a single exon is available for targeted mutation analysis. Patients' sequences are compared with the reference DNA sequence.[11]

This study was conducted in accordance with the revised Declaration of Helsinki (1998) and approved by the Research Ethics Committee of Sakarya University Training and Research Hospital. A written informed consent was obtained from all participants.

Statistical Analysis

Statistical analysis of this study was performed using the Statistical Package for Social Sciences (SPSS) program (18.0 Windows; SPSS Inc., Chicago, IL). Descriptive statistics were calculated for all continuous and categorical variables to enable a thorough description of the demographic, clinical, and radiological characteristics of the patients. Continuous data are described by mean ± standard deviation or median and interquartile range. Categorical data are presented as numbers or percentages.

Results

A total of 484 young patients (aged 18-55) were admitted to the emergency department with acute ischemic attack. Patients with atrial fibrillation (n: 81), other cardiovascular diseases (n: 48), acute myocardial infarction