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Fabry disease screening in kidney transplant patients: A single-center study in Türkiye

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ABSTRACT

Aims: This study aimed to screen for Fabry disease in adult kidney transplant patients at a nephrology clinic in Türkiye.

Methods: This cross-sectional, single-center study prospectively enrolled kidney transplant recipients regardless of the etiology of renal failure. α-galactosidase A (α-GLA) enzyme activity and α-GLA gene analysis were used to screen for Fabry disease. The screening was initiated by measuring enzyme activity in males, and those with <2.5 nmol/mL/hour activity underwent gene analysis. Females were screened directly by gene analysis, independent of the enzyme activity.

Results: We screened 125 patients (age: 48.9±10.1, male: 70.4%). Gene analysis was performed on a 68-year-old male patient with enzyme activity at the lower end of the reference range. No mutations associated with Fabry disease were detected. The enzyme activity test was considered false positive. A heterozygous c.937G>T (p. D313Y) mutation was detected in the gene analysis of a 29-year-old female patient. However, systemic evaluation did not reveal any clinical findings consistent with Fabry disease. Screening tests were within normal limits in other patients. Although there were abnormal screening findings in 2 patients, none was diagnosed with Fabry disease.

Conclusions: Screening studies for Fabry disease in kidney transplant patients may contribute to the determination of the true prevalence.

Introduction

Fabry disease, also called Anderson-Fabry disease, is a rare lysosomal storage disorder carried on the X chromosome. Pathogenic mutations diminish the activity of the enzyme α-galactosidase A (α-GAL-A) and lead to the accumulation of substrates, such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Endothelial deposits that progress over the years result in progressive organ destruction and failure, particularly in renal, cardiac, and cerebrovascular cells (1-3).

The prevalence of the disease is reported in the general population between 1: 40,000 and 1: 117,000 (1,4). Non-specific clinical findings and a relatively slow rate of progression make it difficult to identify the patients despite obvious clinical symptoms. Moreover, a study from Türkiye has shown that physicians were not adequately familiar with the clinical signs and symptoms of Fabry disease, which may cause delayed diagnosis (5). Thus, screening studies in high-risk groups may help identify undiagnosed patients.

The results of studies conducted on kidney transplant patients declare that Fabry disease is detected more frequently in this group than in the general population (6-11). In a few studies conducted in our society, the prevalence varies from 0.09% to 0.5% (8-10). Therefore, kidney transplant patients can

be considered a risky population. Screening these patients may enable the diagnosis of formerly undiagnosed cases and perhaps some patients among their family members. In all identified cases, with or without a kidney transplant, specific treatments such as enzyme replacement and chaperone treatments that can offset some multisystemic effects of the disease may come to the fore (12,13).

This study aimed to screen for Fabry disease in adult kidney transplant patients at a nephrology clinic in Türkiye.

Methods

Study design

This was a cross-sectional, prospective, single-center screening study designed to screen for Fabry disease in kidney transplant patients at the University of Health Sciences Türkiye, Diskapi Yildirim Beyazit Training and Research Hospital, Nephrology Clinic, Ankara, Türkiye. The Local Ethics Committee (protocol no: 64/10, date: 28.05.2019) approved the study protocol. All participants provided written informed consent. The study protocol followed the principles of the revised version of the Declaration of Helsinki.

All kidney transplant patients aged 18 years or older under follow-up with functional grafts in our institution were deemed eligible for the study. A male patient diagnosed with Fabry disease before the initiation of the study was excluded. Patients who agreed to participate were included in the study independent of signs, symptoms, or family history, even if a primary kidney disease had already been recorded. There was no intervention in the routine treatment and follow-up of the patients. Demographic data included age, gender, etiology of kidney disease, and the date of transplantation.

Screening protocol

α-GAL-A enzyme activity and α-GLA gene analysis were used to screen for Fabry disease. The screening was started by measuring enzyme activity in men, and gene analysis was planned for those with <2.5 nmol/mL/hour. Since the sensitivity and specificity of enzyme activity measurement results in females were below 50%, screening was performed with α-GLA gene analysis (4). A detailed clinical work-up that included cardiologic, neurologic, dermatologic and ophthalmologic examinations was prepared for the individuals diagnosed with Fabry disease by screening tests.

α-GAL-A enzyme activity analysis

α-GAL-A enzyme activity was measured in dry blood samples by the method described by Chamoles et al. (14). Peripheral blood samples taken during the routine examination of the patients were dropped immediately on filter paper [dry blood samples (DBS)], and the three circles on the paper were equally saturated. The paper was dried at room temperature for at least four h and stored at + 4 °C until reaching the Düzen Laboratories, Ankara, Türkiye, within five days. Later, DBS paper was processed by the fluorimetric method. 4-methylumbelliferyl-α-D-galactopyranoside (TRC, M334475) was used as the substrate, and N-acetyl-D-galactosamine (Sigma, A2795) was used as the inhibitor. 3 mm DBS punches were incubated with substrate and inhibitor at 37 °C for 17 h. Fluorescence was recorded in the fluorimeter. The results were examined by constructing a calibration curve with 4-methylumbelliferone (Sigma M1381). For α-GAL-A enzyme activity, values of 0.6 nmol/mL/hour were significant for deficiency, while values above 2.5 nmol/mL/hour were normal.

α-GAL-A gene mutation analysis

α-GAL-A gene mutation analyzes were performed on 3 cc venous blood samples collected in EDTA-containing tubes (stored at +4 ºC, and for a maximum of 5 days). All analyses were performed at the Intergen Genetic Diagnosis Center, Ankara, Türkiye, as described in the literature (15). DNA extracted from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen Inc.) was stored at -20 °C until the polymerase chain reaction (PCR) step. Using PCR primers designed with Primer© - Primer Designer v.2.0 (Scientific and Educational Software), all coding exons of the gene and their splice junctions were amplified. The PCR pool was purified using the NucleoFast® 96 PCR cleanup kit (Macherey-Nagel GmbH). The purified PCR pool was measured using a Nanodrop 1000 microvolume spectrophotometer (Thermo Inc.) and diluted before sequencing. α-GLA gene sequence analysis was performed using the MiSeq NGS (Next Generation Sequencing) platform (Illumina, San Diego, CA, USA). Data were visualized with IGV 2.3 (Broad Institute) software.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences Statistics for Windows, version 22.0 (Armonk, NY: IBM Corp., 2013). The distribution normality for continuous variables was tested using the Kolmogorov-Smirnov test and histograms. Normally distributed data were presented as mean±standard deviation. Categorical variables are shown as frequency and percentage.

Results

The characteristics of the study population are presented in Table 1. We screened 125 kidney transplant patients with a mean age of 48.9±10.1 years and male predominance (n=88, 70.4%). The time after transplantation was 10.1±6.8 years. The underlying kidney disease was unknown in (56%) 70 patients. The remaining 24 (19.2%) subjects had hypertension, 8 (6.4%) had glomerulonephritis, 6 (4.8%) had diabetes mellitus, 6 (4.8%)

had vesicourethral reflux disease, 5 (4%) had pyelonephritis, 3 (2.4%) had kidney stones, 2 (1.6%) had polycystic kidney disease, and 1 (0.8%) had Alport's disease.

The characteristics of two cases with abnormal findings are presented in Table 2. In 1 of 88 male patients, α-GAL-A enzyme activity was detected at the lower end of the reference range (Case 1). α-GLA gene analysis was performed on this patient and no mutations related to Fabry disease were detected. Enzyme measurements of the other males were within the normal range. All female patients (n=37) were screened by gene mutation analysis. A heterozygous mutation of c.937G>T (p.D313Y) was detected in one female patient (Case 2). Gene analysis was normal in the remaining 36 female patients.

Case 1

A 68-year-old male patient had undergone ABO-compatible living kidney transplant 14 years ago. The cause of kidney disease was unknown. The serum creatinine level was approximately 1.3 mg/dL under triple immunosuppressive therapy comprising prednisolone, tacrolimus, and mycophenolate mofetil. Urinalysis was within normal limits. α-GLA gene analysis was performed because the result of enzyme activity, which was the first screening test of this patient, was at the lower limit of normal. No mutations associated with Fabry disease were detected. The enzyme activity test was considered false positive.

Case 2

A 29-year-old female patient with a kidney transplantation history from her mother at another center two months ago was admitted to our clinic for follow-up. She had C.937G>T (p.D313Y) heterozygous mutation on gene analysis. However, there were no clinical finding consistent with Fabry disease for further evaluation. Genetic analysis of her mother showed no mutations. It was considered the mutant allele inherited from her father who was not alive. Of her eight siblings, three had kidney disease of unknown origin. However, they were also all abroad. Although the findings suggested an unexplained familial kidney disease, they were inconsistent with Fabry disease. She is still followed up with stable kidney function and urinalysis.

Table 1. Characteristics of the study population

	Total patients (n=125)
Gender (male, n, %)	88 (70.4)
Age at screening (year)	48.9±10.1
Primary cause of kidney disease (n, %)	
Hypertensive nephropathy	24 (19.2)
Chronic glomerulonephritis	8 (6.4)
Diabetes mellitus	6 (4.8)
Vesicourethral reflux disease	6 (4.8)
Pyelonephritis	5 (4.0)
Nephrolithiasis	3 (2.4)
Polycystic kidney disease	2 (1.6)
Alport disease	1 (0.8)
Unknown	70 (56.0)
Time after transplantation (years)	10.1±6.8

Continuous variables were expressed as mean±standard deviation

Discussion

Nephropathy is one of the most frequent complications of Fabry disease. For this reason, screening chronic kidney disease patients as a relevant risk group for Fabry disease is sound to manage the potential complications earlier. In this study, we screened Fabry disease in kidney transplant patients followed up in our institution, and identified a heterozygous variant mutation in a female patient. However, we were unable to substantiate its clinical implication.

The frequency of Fabry disease in kidney transplant patients is unclear, and screening studies in this population are limited (6-11). In two screening studies, 1 male in 673 (6) and 5 males in 1,306 kidney transplant recipients were diagnosed with Fabry disease (7). Only a few studies have been conducted in Türkiye so far, which have indicated that the prevalence of Fabry disease among kidney transplant patients is above the general population figures. Concerning kidney transplant patients, 1 (0.09%) in 1,095 at Ege University (8), 1 (0.33%) in 301 at Ankara University (9), and 1 (0.5%) in 200 at Haydarpasa Numune Hospital (10) were the carriers of the specific mutation. Although the current study sample was small, it is one of the few studies that have screened Fabry disease in kidney transplant patients in the country, and we have identified no mutation that

Table 2. Characteristics of the cases with abnormal findings

	Case 1	Case 2
Age (year)	68	29
Gender	Male	Female
Donor	Non-relative male	Mother
Primary kidney disease	Unknown	Unknown
α-GAL-A enzyme activity	2.5 nmol/mL/hour	-
α-GLA gene mutation	No mutation	c.937G>T (p.D313Y) heterozygote

α-GLA: α-galactosidase, α-GAL-A: α-galactosidase A

would definitively confirm Fabry disease in any recipient. Existing knowledge suggests that physician awareness of Fabry disease is insufficient in Türkiye, which may delay the diagnosis (5). The patient we excluded from the analysis because Fabry disease was diagnosed before the current study may be an example of a late diagnosis. The patient had Fabry disease diagnosis 6 years after the transplantation. Using a screening program, he could have been accurately diagnosed with a screening test before experiencing a cerebrovascular accident. However, with limited data and practice, it may not be feasible to screen all transplant cases for Fabry disease. However, screening for Fabry disease can be feasible, particularly in kidney recipients with an unknown etiology of kidney damage and whose family members have kidney disease.

In this study, one of the two patients with an abnormal result was a male patient whose α-GLA gene analysis was performed because α-GAL-A enzyme activity was at the lower limit of normal. No pathological mutation was detected in the gene analysis of this patient. A similar finding was previously found in a screening study on hemodialysis patients, in which no gene mutations were detected despite low enzyme activity in 29 of 526 patients (16). The authors suggested that malnutrition and chronic inflammation, common in dialysis patients, have led to false positive test results by impairing protein synthesis (16). In our study, only one of 88 patients had a false-positive result. This patient was on antibiotic therapy for a urinary tract infection when the blood sample was collected, suggesting that acute inflammation affected the test result. Performing enzyme activity analyses in stable periods can prevent unnecessary loss of time and cost.

In this study, the c.937G>T (p.D313Y) mutation was detected in the genetic analysis of a female patient. This is a variant whose pathogenicity was questioned after a second mutation was detected in further analysis of the genetic material of the patient in which it was first identified (17). It has been shown that the activity of the enzyme in patients carrying this mutation is reduced at neutral pH (7.4) but stabilized at lysosomal pH (4.6). With this finding, it was interpreted that this mutation may cause a false deficiency in plasma α-GAL-A activity (18). These data are also supported by a meta-analysis of 35 recent clinical studies. High residual enzyme activities and normal lyso-Gb3 concentrations were detected in patients with the D313Y genotype without Gb3 deposits. A striking point is the higher prevalence of this variant in patients with neurological disorders (19). However, more studies are needed to clarify its relationship with neurological findings. The prevalence of D313Y in the general population is around 0.5% (18). In this study, it was detected in only one patient and its prevalence was calculated as 0.79%. However, in this study, male patients were initially screened with enzyme activity analysis. Considering that enzyme activity may not be decreased in D313Y carriers, male patients with mutations may have been overlooked. As a result, D313Y was classified as a neutral variant of unknown significance based on the available data. There were no additional findings consistent with Fabry disease in the patient we display in this study. Although a native kidney biopsy could not be performed, the obtained data did not support the diagnosis of Fabry disease.

Study Limitations

This study has some limitations. Since it was a single-center study, the number of participants was limited. Also, the family members of patient 2 remained unexamined except for the mother.

Conclusion

In conclusion, screening studies for Fabry disease in kidney transplant patients may contribute to the determination of the true prevalence and allow diagnosis before some complications develop.

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Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, Diskapi Yildirim Beyazit Training and Research Hospital Local Ethics Committee (protocol no: 64/10, date: 28.05.2019).

Informed Consent: All participants provided written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.M.A., G.U.O., T.S., K.G.A., M.D.A., Concept: Ş.M.A., G.U.O., M.D.A., Design: Ş.M.A., G.U.O., Data Collection or Processing: Ş.M.A., G.U.O., K.G.A., Analysis or Interpretation: Ş.M.A., G.U.O., Literature Search: Ş.M.A., G.U.O., T.S., Writing: Ş.M.A., G.U.O.

Conflict of Interest: All authors declare that they have no conflict of interest.

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