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Original Article

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Screening for the Frequency of Fabry Disease in Patients Followed Up in the Nephrology Outpatient Clinic

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ABSTRACT

Background: The goal of this study was to determine the prevalence of Fabry disease in the nephrology outpatient clinic population, whose diagnosis is frequently delayed despite the availability of enzyme replacement therapy today. Additionally, it was aimed at identifying patient groups that should be evaluated in terms of Fabry disease diagnosis based on symptoms, making an early diagnosis in some patients through family screening, and starting early treatment in these patients.
Methods: The study, which enrolled 950 patients, followed in our outpatient clinic. Questionnaires were used to assess the patients. According to the results of the questionnaire, blood samples were taken from those with the possibility of Fabry disease, and the disease was screened by examining Fabry gene mutations in female patients and alpha-galactosidase A levels in male patients.
Results: A female patient with Fabry disease was not included in the study. During the study only one female patient was found to have a genetic mutation. The diagnostic phase of the patient, who had a genetic variant considered as a polymorphism by some authors, could not be completed because the diagnosis of amyloidosis-myeloma was made and the patient died without further examination.
Conclusion: Although individuals with the possibility of Fabry disease were selected by a questionnaire study in a specific patient population, the frequency of the disease was found to be consistent with the literature.
Keywords: Fabry disease, chronic kidney failure, genetic disease

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INTRODUCTION

Fabry disease is an X-linked recessive disease with multisystem involvement caused by a decrease in the activity of the alpha-galactosidase A (GLA) enzyme.[1] The main pathology causing the disease is the accumulation of glycosphingolipids in the lysosomes as a result of inadequate enzyme activity.[1] Patients with classic Fabry disease have less than 1% of normal plasma-galactosidase activity and are typically male due to X-linked inheritance.[2] Because it is a rare disease, it is frequently misdiagnosed or underdiagnosed. Although the exact incidence and prevalence are unknown, it is estimated in the general population to be between 1 in 8000 and 1 in 120 000.[3] Because of its life-threatening features, such as cerebrovascular accident, kidney failure, cardiomyopathy, arrhythmias, left ventricular hypertrophy, and heart attack at an early age, as well as the presence of symptoms that impair the quality of life, such as hearing loss, tinnitus, fatigue, abdominal pain, diarrhea, dry skin, hot and cold exercise intolerance, visual impairment, angiokeratomas, peripheral neuropathy, and the inability to sweat, early detection of Fabry disease is critical.[4] Low enzyme levels in males and normal enzyme levels in heterozygous females can be seen after a high clinical suspicion and a thorough physical examination, and the diagnosis is confirmed

by genetic testing.[2] Unfortunately, many patients are evaluated by multiple specialists before being diagnosed, and the average time for diagnosis is 13-16 years.[4]

The purpose of this study was to look for the presence of Fabry disease in patients whose cause of kidney disease could not be determined in the nephrology outpatient clinic and who had other unresolved complaints in addition to kidney disease. Our secondary goal was to conduct family screenings on individuals diagnosed with Fabry disease in order to determine whether this hereditary disease affects other family members and, if so, to receive treatment.

MATERIAL AND METHODS

The study was performed on patients who were followed up regularly in the nephrology outpatient clinic. Patients diagnosed with chronic kidney disease (CKD) according to the Kidney Disease: Improving Global Outcomes 2012 guideline were included in the study. Chronic kidney disease is defined as the presence of kidney damage (usually detected as urinary albumin excretion of ≥ 30 mg/day or equivalent) or decreased kidney function (defined as estimated glomerular filtration rate [eGFR] < 60 mL/min/1.73 m^2) for 3 or more months, irrespective of the cause. A total of 950 patients aged between 18 and 80 years were included in the study. Among these patients were patients with additional diseases such as cerebrovascular disease, peripheral vascular disease, diabetes mellitus, hypertension, and coronary artery disease. Additional diseases among the patients were recorded. A group of patients also had acute kidney injury on the basis of CKD. These patients were also registered. The patients' creatinine levels, proteinuria values, and whether they had hematuria were recorded. For microscopic hematuria, a direct red blood cell (RBC) count per mL of uncentrifuged urine was performed. Microscopic hematuria was defined as 3 or more RBCs per high-power field.

The patients were informed about Fabry disease on the day of the outpatient clinic appointment. Informed consent forms were filled out by those who volunteered to participate in the study. A survey with 11 questions was used for screening purposes. This questionnaire was created by examining studies conducted for Fabry disease screening and reviewing the literature.[5,6] The questions have been revised in a way that our patient population can understand. Patients were assisted in answering the questions correctly.The survey included the name of the study and the date the survey was completed. In question 1, there were two photographs with angiokeratomas. The patients were asked whether they had these and similar skin lesions. In question 2, the patients were asked about the presence of CKD. In question 3, they were asked about the presence of CKD in the family. In question 4, they were asked about stroke history. In question 5, they were asked about a family history of stroke before the age of 50 years. In question 6, the patients were asked about history of heart disease (arrhythmia, coronary artery disease, myocardial infarction). In question 7, they were asked about the presence of any of these diseases in a family member before the age of 50 years. In question 8, they were asked about inability to sweat and decrease in sweating. In question 9, they were asked about complaints such as pain, a feeling of electric shock, tingling, and numbness that started in the arms and legs, which spread to the rest of the body, worsened with heat or cold, and continued since childhood. In question 10, the patients were asked about the presence of individuals diagnosed with Fabry disease in the family. The first 10 questions were answered yes or no. In question 11, they were asked about other features of Fabry disease; the patients were questioned about hearing loss, tinnitus, balance disorder, dizziness, headache, forgetfulness, cataract, inability to gain weight, blindness, osteoporosis, unexplained constant fatigue, excessive sweating, intolerance to heat or cold, dry skin, unexplained abdominal pain, and diarrhea lasting longer than 1 month. Patients who answered "yes" to questions 1, 4, and 6 were scheduled for testing regardless of other signs and symptoms. The test was planned for those who answered "yes" to 2 of the first 10 questions. The test was planned for the patients who answered "yes" to 1 of the first 10 questions and answered with 4 or more "yes" to the 11th question.

The ERBP guideline (2012) does not recommend screening males with an age higher than 50 if they have unexplained CKD. However, some patients over the age of 50 were also tested for reasons such as an unexplained stroke. Genetic mutation analysis was performed in women and GLA enzyme activity in men. Fabry disease mutations were investigated in male patients with GLA enzyme activity below 2.5 nmol/mL/hour, just like in female patients. Analysis of the mutations identified for Fabry disease was compared with the NCBI genomic reference sequences (NG_007119.1 and NM_000169.2), and genomic DNA sequence analysis in the GLA gene (exon 1-7) was performed. Approval for the study was obtained from the local Clinical Research Ethics Committee of Ege University (Date: February 20, 2018, Protocol No:18-2.1/23). The researchers stated that it would be carried out in accordance with the current Helsinki Declaration and good clinical practice principles. The Statistical Package for the Social Sciences Statistics software, version 22.0 (IBM SPSS Corp.; Armonk, NY, USA), was used for statistical analysis. Differences between gender groups were demonstrated by 2 proportion tests. Detailed analyses between groups were not possible, as a patient with a gene mutation could be found in the study.

MAIN POINTS

- High-risk patients were identified using a questionnaire in this study, and the frequency of Fabry disease in patients followed in the nephrology outpatient clinic was investigated using genetic analysis.
- Only 1 patient had a target mutation, but she was not diagnosed with Fabry disease.
- This study confirms the rarity of Fabry disease, even in specific groups, and paves the way for larger-scale clinical trials.

RESULTS

Of the patients, 424 (44.6%) were female and 526 (55.3%) were male. The mean age of the patients was 58 years, 57 for women and 61 for men. The clinical features of the patients are shown in Table 1. When the data files of 950 patients who participated in the survey were examined retrospectively, it was determined that 286 (30.1%) patients underwent kidney biopsy. The most common diagnosis was focal segmental glomerulosclerosis (FSGS), accounting for 45.4% of all biopsies (Table 2). Fabry disease was investigated by enzyme level or gene mutation in all patients with focal segmental glomerulosclerosis, but no gene mutation was found. Electron microscopy could not be performed on any of our patients due to technical limitations. On the other hand, kidney biopsies of 13 (approximately 10%) patients diagnosed with FSGS were reevaluated retrospectively. However, because the thin section technique was not used, no evidence supporting Fabry disease could be obtained. Additional diseases of the patients are shown in Table 3. Chronic kidney disease is most often accompanied by hypertension and diabetes mellitus. The answers given to the survey questions are given in Table 4. According to the results of the questionnaire, blood samples were taken from 329 (34%) patients who were planned to be tested for Fabry disease. Genetic mutation analysis was performed on 165 female patients (38%) whose blood samples were taken. A heterozygous c.937G>T (p.D313Y) (p.Asp313Tyr) mutation in the GLA gene (NM_0000169.2) was detected in only 1 female patient (0.7%). On the other hand, GLA enzyme activity was measured in 164 male patients (34%) whose blood samples were taken. Gene mutation analysis was performed with a preliminary diagnosis of Fabry disease in 33 male patients (20%) with a level of ≤ 2.5 nmol/mL/hour, and no known mutation was detected. Also, 1 patient with a diagnosis of Fabry disease was excluded from the study.

Table 1. The Ratio of Hematuria, Creatinine Value Range, and Proteinuria Value Ranges in Female and Male Patients to all Patients

	Total (n = 950)	Female (n = 424)	Male (n = 526)	
	(100%)	(44.6%)	(55.3%)	*P*
Hematuria				
Negative	797 (83.8%)	347 (81.8%)	450 (85.5%)	.125
Positive	153 (16.1%)	77 (18.1%)	76 (14.4%)	.125
Creatinine				
≥ 3 mg/dL	156 (16.4%)	60 (14.1%)	96 (18.2%)	.086
1.-3 mg/dL	368 (38.7%)	124 (29.2%)	244 (46.3%)	.001
≤ 1.3 mg/dL	426 (44.8%)	240 (56.6%)	186 (35.3%)	.001
Proteinuria				
≥ 3 g/dL	70 (7.3%)	29 (6.8%)	41 (7.7%)	.573
0.5-3 g/dL	275 (28.9%)	111 (26.1%)	164 (31.1%)	.089
≤ 0.5 g/dL	605 (63.6%)	284 (66.9%)	321 (61%)	.056

Table 2. Biopsy Results of Patients Who Underwent Kidney Biopsy According to Gender

	Total (n = 950)	Female (n = 424)	Male (n = 526)	
	(100%)	(44.6%)	(55.3%)	*P*
Biopsies	286 (30.1%)	129 (30.4%)	157 (29.8%)	.847
FSGS	130 (45.4%)	64 (15%)	66 (12.5%)	.260
IGA	56 (19.5%)	21 (4.9%)	35 (6.6%)	.261
MGN	55 (19.2%)	24 (5.6%)	31 (5.8%)	.878
Cancer	13 (4.5%)	5 (1.1%)	8 (1.5%)	.648
MPGN	12 (4.1%)	8 (1.8%)	4 (0.7%)	.139
TIN	2 (0.6%)	0 (0%)	2 (0.3%)	.157
MCD	2 (0.6%)	0 (0%)	2 (0.3%)	.157
Normal	1 (0.3%)	1 (0.2%)	0 (0%)	.317
Insufficient material	2 (0.6%)	0 (0%)	2 (0.3%)	.157

FSGS, focal segmental glomerulosclerosis; IGA, IgA nephropathy; MCD, minimal change disease; MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; TIN, tubulointerstitial nephritis.

DISCUSSION

Fabry disease is a difficult disease to recognize and should be kept in mind in the differential diagnosis in order to detect it early. Many patients have been reported who were misdiagnosed or took years to be diagnosed.[1] Given that there is a cure for this disease, early diagnosis becomes critical.

Table 3. Known Diseases of the Study Participants

	Total (n = 950)	Female (n = 424)	Male (n = 526)	
	(100%)	(44.6%)	(55.3%)	*P*
Hypertension	498 (52.4%)	221 (52.1%)	277 (52.6%)	.869
Diabetes mellitus	192 (20.2%)	78 (18.3%)	114 (21.6%)	.208
Coronary artery disease	111 (11.6%)	42 (9.9%)	69 (13.1%)	.120
Glomerulonephritis	99 (10.4%)	51 (12%)	48 (9.1%)	.150
Connective tissue disease	63 (6.6%)	34 (8%)	29 (5.5%)	.129
Malignancy	47 (4.9%)	14 (3.3%)	33 (6.2%)	.030
Solitary kidney	28 (2.9%)	12 (2.8%)	16 (3%)	.847
Cerebrovascular disease	28 (2.9%)	8 (1.8%)	20 (3.8%)	.072
Liver failure	18 (1.8%)	7 (1.6%)	11 (2%)	.616
Peripheral vascular disease	16 (1.6%)	7 (1.6%)	9 (1.7%)	.943
Acute kidney failure	8 (0.8%)	6 (1.4%)	2 (0.3%)	*p*.102

Due to the difficulty in diagnosing Fabry disease, screening tests and case-finding studies are frequently conducted in patient groups with risk factors. Moreover, the frequency of Fabry disease is increasing in studies conducted in risk groups. Since patients followed for kidney disease are at risk, Fabry disease screening was planned in this patient population in our study, and a questionnaire was applied to patients followed in the nephrology outpatient clinic to reduce the cost. Based on questionnaire studies in the literature, a new questionnaire was developed for our study and adapted for the patients. According to our results, enzyme activity and gene analysis were performed on these chronic kidney failure patients, and genetic variation in 1 female patient (0.4%) and low GLA levels in 25 male patients (6%) were found.

In all patients with focal segmental glomerulosclerosis, Fabry disease was investigated by enzyme level or gene mutation, but no gene mutation was found. GLA activity was investigated in 62 patients with biopsy-proven FSGS in a study and low enzyme activity was found in 9% of the patients.[9] Patients who had low enzyme levels but could not be diagnosed with Fabry disease were explained by protein loss due to podocyte damage.[9]

In only 1 female patient (0.7%), a heterozygous c.937G>T (p.D313Y) (p.Asp313Tyr) mutation was detected in the GLA gene (NM_0000169.2). The D313Y mutation is a controversial mutation related to Fabry disease. While some authors suggest that it may cause the disease, others believe it is a genetic polymorphism that does not cause the disease.[10] When the literature is examined, there are some studies reporting a 60% decrease in enzyme activity as a result of this mutation.[2] However, Fabry disease was not found in more than half of the patients with low enzyme activity and the D313Y mutation. This mutation, on the other hand, was detected in 25% of patients with a cryptogenic cerebrovascular history.[11] In patients with hypertrophic cardiomyopathy, the D313Y mutation was observed at a rate of 33%.[12] Similarly, in the screening studies of hemodialysis patients, the presence of the mutation was reported as 28%.[3] However, the D313Y mutation is listed as a single-nucleotide polymorphism in the Single Nucleotide Polymorphism Database, and it has been reported that its frequency may be up to 1% in populations.[13] In our study, the patient with this mutation had a history of migraines, hypothyroidism, and diabetes mellitus. Although this 58-year-old female patient had leg edema and proteinuria of 11 g/day on examination, kidney failure was not detected at the time of initial diagnosis. A kidney biopsy was performed with the diagnosis of nephrotic syndrome, and non-AA amyloidosis was found in the patient. During the follow-up, a bone marrow biopsy was performed, and the patient was diagnosed with amyloidosis associated with multiple myeloma based on the results of the biopsy. The hematology department evaluated the patient and the vincristine, doxorubicin, and dexamethasone regimen was initiated.

The frequency of gene mutations for Fabry disease was found to be as high as one in 150 in questionnaire studies conducted in isolated left ventricular hypertrophy cardiomyopathy and cryptogenic cerebrovascular disease groups.[14] The reason for the absence of mutations in our study can be explained by a lack of patients and the inability of patients to fully answer questions about the etiology of cardiac and cerebrovascular diseases during the questionnaire. According to the questionnaire questions, 350 patients were tested. However, neither enzyme deficiency nor gene mutation were detected in patients who answered these questions positively. The presence of the questioned symptoms in these patients was attributed to other chronic diseases.

It is difficult to diagnose Fabry disease, but the important point to remember is that it can coexist with other diseases. Therefore, Fabry disease should always be kept in mind. The mutation found in only 1 patient in our study can be attributed to many reasons. First of all, it has been confirmed that Fabry disease is very rare. More patients should be tested to find such a rare disease. The questionnaire used should be developed and validated.

In conclusion, patients with kidney disease who were regularly followed up in the nephrology outpatient clinic were examined in this study, and enzyme and gene analyses were performed using a questionnaire in patients with signs and symptoms suggestive of Fabry disease. While a patient with a diagnosis of Fabry disease was not included in the study, a genetic mutation was found in one patient. The diagnosis stage of the patient, who had a genetic variant considered a polymorphism by some authors, could not be completed due to the additional diagnosis of amyloidosis associated with myeloma, the refusal of the family members to examine the patient, and the patient's death without further investigation in the follow-up.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ege University (Date: February 20, 2018, Protocol No:18-2.1/23).

Informed Consent: Written informed consent was obtained from E.K., the patient with the mutation, and all other patients participating in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.Y.; Design – M.Y.; Supervision – M.Y.; Resources – M.Y., S.S.; Materials – S.S.; Data Collection and/or Processing – S.S., G.A., M.S.D., A.Ç., G.A., M.Y.; Analysis and/or Interpretation – S.S., M.Y.; Literature Search – S.S., M.Y.; Writing Manuscript – S.S., M.Y.; Critical Review – M.Y.

Declaration of Interests: The authors have no conflict of interest to declare.

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REFERENCES

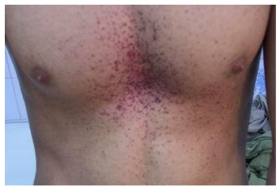
1. Bokhari SRA, Zulfiqar H, Hariz A. Fabry disease. In: *StatPearls*. Treasure Island FL: StatPearls Publishing LLC; 2023.
2. Mahmud HM. Fabry's disease--a comprehensive review on pathogenesis, diagnosis and treatment. *J Pak Med Assoc*. 2014;64(2):189-194.
3. Bernardes TP, Foresto RD, Kirsztajn GM. Fabry disease: genetics, pathology, and treatment. *Rev Assoc Med Bras (1992)*. 2020;66(suppl 1):s10-s16. [CrossRef]
4. Mehta A, Ricci R, Widmer U, et al. Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. *Eur J Clin Investig*. 2004;34(3):236-242. [CrossRef]
5. Arning K, Naleschinski D, Maag R, et al. FabryScan: a screening tool for early detection of Fabry disease. *J Neurol*. 2012;259(11):2393-2400. [CrossRef]
6. Sodré LSS, Huaira RMNH, Bastos MG, Colugnati FAB, Coutinho MP, Fernandes NMDS. Screening for Fabry disease in kidney disease: a cross-sectional study in males and females. *Kidney Blood Press Res*. 2017;42(6):1258-1265. [CrossRef]
7. Terryn W, Cochat P, Froissart R, et al. Fabry nephropathy: indications for screening and guidance for diagnosis and treatment by the European Renal Best Practice. *Nephrol Dial Transplant*. 2013;28(3):505-517. [CrossRef]
8. Choi JH, Lee BH, Heo SH, et al. Clinical characteristics and mutation spectrum of GLA in Korean patients with Fabry disease by a nationwide survey: underdiagnosis of late-onset phenotype. *Med (Baltim)*. 2017;96(29):e7387. [CrossRef]
9. Hasbal NB, Caglayan FB, Sakaci T, et al. Unexpectedly high prevalence of low alpha-galactosidase A enzyme activity in patients with focal segmental glomerulosclerosis. *Clinics (Sao Paulo)*. 2020;75:e1811. [CrossRef]
10. Yasuda M, Shabbeer J, Benson SD, Maire I, Burnett RM, Desnick RJ. Fabry disease: characterization of alpha-galactosidase A double mutations and the D313Y plasma enzyme pseudodeficiency allele. *Hum Mutat*. 2003;22(6):486-492. [CrossRef]
11. Baptista MV, Ferreira S, Pinho-E-Melo T, et al. Mutations of the GLA gene in young patients with stroke: the PORTYSTROKE study–screening genetic conditions in Portuguese young stroke patients. *Stroke*. 2010;41(3):431-436. [CrossRef]
12. Monserrat L, Gimeno-Blanes JR, Marín F, et al. Prevalence of Fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2007;50(25):2399-2403. [CrossRef]
13. Gaspar P, Herrera J, Rodrigues D, et al. Frequency of Fabry disease in male and female haemodialysis patients in Spain. *BMC Med Genet*. 2010;11:19. [CrossRef]
14. Lenders M, Duning T, Schelleckes M,et al. Multifocal white matter lesions associated with the D313Y mutation of the α-galactosidase A gene. *PLOS ONE*. 2013;8(2):e55565. [CrossRef]

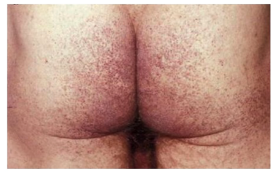
QUESTIONNAIRE

Name of the Research: SCREENING FOR THE FREQUENCY OF FABRY DISEASE IN PATIENTS FOLLOWED UP IN THE NEPHROLOGY OUTPATIENT CLINIC

Case number: **Date:**

1) Do you have lesions on your skin similar to those in the picture? YES NO





2) Do you have protein leakage loss in your kidney or kidney failure?

YES NO

3) Do you have any relatives who have kidney failure or protein leakage loss from their kidneys?

YES NO

4) Have you had a stroke? YES NO

5) Is there anyone in your family who had a stroke before the age of 50? YES NO

6) Do you have heart disease, an enlarged heart, a heart attack history, or arrhythmia?

YES NO

7) Is there anyone in your family who has had a heart disease or had a heart attack before the age of 50?

YES NO

8) Do you have complaints of inability to sweat or decrease in sweating?

YES NO

9) Do you have complaints such as pain, electricity, tingling, or numbness that begins in your arms or legs, spreads to the rest of your body, worsens with heat or cold, and has persisted since your childhood?

YES NO

10) Is there anyone in your family who has Fabry Disease?

YES NO

11) Please check any of the boxes that apply to you.

- □ Bone resorption
- □ Unexplained persistent fatigue
- □ Excessive sweating
- □ Intolerance to heat or cold
- □ Skin dryness
- □ Unexplained abdominal pain
- □ Diarrhea lasting longer than 1 month
- □ Accumulation of substance in the eye
- □ Hearing loss
- □ Tinnitus
- □ Balance disorder
- □ Dizziness
- □ Headache
- □ Forgetfulness
- □ Cataract
- □ Not gaining weight
- □ Blindness