DOI: https://doi.org/10.2298/SARH211103037C
UDC: 616-056.7:616.61-78-056.24(497.113)

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

High risk population screening for Fabry disease in hemodialysis patients in Vojvodina – pilot study

Dejan Ćelić[1,2], Sonja Pavlović[3], Anita Skakić[3], Jelica Vučenović[4], Dragana Pilipović[5], Sonja Golubović[1,2], Tatjana Stojšić[6], Olivera Milićević[7], Goran Vojinović[8], Svetlana Dudić[9], Lada Petrović[1,2], Igor Mitić[1,2]

[1]Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[3]University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia;
[4]Sremska Mitrovica General Hospital, Hemodialysis Center, Sremska Mitrovica, Serbia;
[5]Dr Mladen Stojanović Primary Health Care Center, Hemodialysis Center, Bačka Palanka, Serbia;
[6]Subotica General Hospital, Hemodialysis Center, Subotica, Serbia;
[7]Kikinda General Hospital, Hemodialysis Center, Kikinda, Serbia;
[8]Pančevo General Hospital, Hemodialysis Center, Pančevo, Serbia;
[9]Jovan Jovanović Zmaj Primary Health Care Center, Hemodialysis Center, Stara Pazova, Serbia

SUMMARY

Introduction/Objective Fabry disease (FD) is an X-linked lysosomal storage disease that develops as a consequence of mutation in the alpha-galactosidase A (*GLA*) gene. There are more than 1080 known variants in the *GLA* gene. Some of them are pathogenic, but most of them are benign or represent the genetic change that can be classified as a genetic variant of unknown significance or simply be a representation of genetic polymorphism. There are two main features of FD, classic form and late-onset variants of disease. The main target organs in patients with FD are the kidneys, heart, and nervous system. Bearing in mind the fact that FD is a rare disease, the best way for active searching of patients is high-risk population screening, after which family screening for every proband case should be performed.

Methods In this paper, we present results of a multicentric pilot study that represents findings from the screening of hemodialysis patients for FD in six hemodialysis units in Vojvodina.

Results We have found one patient with benign mutation and 16 patients with genetic polymorphisms in *GLA* gene. We have learned that genetic changes in *GLA* gene can be frequent, but very rarely are of clinical significance and lead to manifestations of FD.

Conclusion Results of this screening study will give us important insights into our future work.

Keywords: Fabry disease; hemodialysis; high-risk population screening

INTRODUCTION

Fabry disease (FD) (OMIM 301500) is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene that result in markedly reduced or absent activity of the enzyme α-galactosidase A (α-Gal A, EC 3.2.1.22). This leads to the intracellular accumulation of substrates like globotriaosylceramide (Gb3) and also its deacylated derivative globotriaosylsphingosine (lyso-Gb3). Lyso-Gb3 is a valuable biomarker and speaks mainly about the burden of FD, since it reflects the severity of disease and also corresponds well with tissue accumulation of Gb3. So, Lyso-Gb3 is of great importance in establishing the diagnosis of FD, but also in assessing the disease severity and therapeutic monitoring [1–4]. Until now, more than 1080 variants in the *GLA* gene have been identified in the Human Gene Mutation Database (HGMD) [5]. Some of them are pathogenic but others are benign or represent genetic change that can be classified as a genetic variant of unknown significance. After the findings of genetic change in *GLA* gene in each patient, we can scroll through the HGMD and seek information about the clinical significance of found mutation [1, 5, 6].

Prevalence of FD is in the range of 1:40,000–1:117,000 live male newborns, but the exact prevalence is very hard to establish due to the very heterogeneous nature of the disease (even within a group of patients with the same genetic variant) and lack of awareness about the disease [7]. In contrast to other X-linked diseases, in which females can only be carriers, in FD females may be as severely affected as male patients [8]. There are two basic phenotypes of FD: a classic form of the disease that is mainly associated with deletions, frameshifts and nonsense variants in the *GLA* gene, and late-onset variants of FD that are associated with missense variants in the *GLA* gene [9]. The classic form of FD starts at a younger age with earlier manifestations on target organs (the heart, kidneys, nervous system) and usually with some other clinical presentations of the disease, like cornea verticillata, angiokeratomas and acroparesthesia (burning sensations on hands and feet). On the other hand, later-onset variants are becoming clinically evident later in life, with cardiomyopathy and/or nephropathy alone [1]. Diagnostic

Received • Примљено: November 3, 2021
Revised • Ревизија: March 30, 2022
Accepted • Прихваћено: April 2, 2022
Online first: April 6, 2022

Correspondence to:
Dejan ĆELIĆ
Hajduk Veljkova 1–7
21000 Novi Sad
Serbia
dejan.celic@mf.uns.ac.rs;
celic.dej@gmail.com

criteria for FD require the confirmation of low enzyme activity and presence of genetic variants in the *GLA* gene for male patients or *GLA* gene variants for female patients together with characteristic clinical features of the disease and/or a family member with the diagnosis of FD and/or elevated plasma lyso-Gb3 level [10]. In some cases of suspected FD target organ biopsy should be performed, with electron microscopy analysis of the tissues, so typical cellular inclusions, "zebra bodies," can be found [1, 2].

The current mainstay of treatment for FD patients includes the administration of enzyme replacement therapy. Nowadays, in clinical practice, there are two available forms of the drug: agalsidase alpha and agalsidase beta, with different dosage regimes (0.2 mg/kg intravenously every other week and 1 mg/kg intravenously every other week, respectively) but similar therapeutic efficacy. In some countries, there is also a possibility for oral therapy with migalastat for some types of amenable mutations [11].

There are only six established cases of FD in Serbia, for now. The exact number of FD patients in our country is hard to establish, but should probably be in the range of 50–100 patients. One way of finding of FD patients is high-risk population screening which represents an active search for FD patients in certain patient populations like hemodialysis patients, patients with hypertrophic cardiomyopathy of unknown/uncertain origin or patients with an early stroke (before the age of 55).

FD often remains undiagnosed according to the prevalence calculated in some studies because of the heterogeneous nature of its clinical manifestations. The clinical diagnosis of both phenotypes (classic form and late-onset variants) is challenging, since many of the main symptoms and signs are common in other diseases, and the time between the first symptoms and the settling of diagnosis can take more than ten years [1, 10]. Therefore, the precise establishment of the molecular-genetic diagnosis and the earliest possible treatment is essential to avoid significant disease progression. Molecular analysis of FD is also crucial for segregation studies, enabling early diagnosis of family members with pathogenic variants in the *GLA* gene allowing monitoring before the first symptoms appear, and therefore promoting better disease management.

The aim of this study was to perform molecular-genetic analysis of FD in the group of hemodialysis patients to provide an early application of appropriate therapy as well as the provision of genetic advice to families with a high risk for the birth of a child with FD.

METHODS

After the approval of ethic committees from every institution which participated in the screening program and the signing of full informed consent by every patient who participated in the study, we performed a multicentric pilot study from December 2020 until May 2021. The study was based on a FD screening of patients in six hemodialysis centers from different parts of Vojvodina (Stara Pazova, Pančevo, Kikinda, Subotica, Bačka Palanka and Sremska Mitrovica). At the time of the screening process, these hemodialysis centers had a dialysis population of 529 patients in total (58, 135, 82, 70, 64, and 120, respectively). Patients eligible for screening were male patients under 55 years of age and with an unknown cause of end-stage renal disease (ESRD), as well as female patients of all ages and unknown cause of ESRD. Because of the variable clinical presentations of FD we analyzed the presence of other clinical manifestations, besides ESRD, that can be a part of the clinical spectrum of FD in our patient eligible for screening process (left ventricular hypertrophy (LVH), stroke before the age of 55, existence of white matter lesions, cornea verticillata, angiokeratoma, tinnitus, hearing loss, heat or cold intolerance, postprandial pain, acroparesthesia, hypo-, or hyperhidrosis). The screening protocol for FD was based upon genetic analysis of peripheral blood from 117 selected patients. Genetic analysis was performed at the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade.

In patients with genetic polymorphisms or genetic variants of unknown significance in the *GLA* gene, we performed biomarker (Lyso-Gb3) analysis in Centogene (Centogene Rostock, Germany) from the blood of our participants through dried blood spots (DBS, CentoCard, Centogene) analysis with the liquid chromatography mass spectrometry method (HPLC-MS/MS; Thermo Fisher Scientific, Waltham, MA, USA).

Detection of genetic variants in the GLA gene

Genomic DNA was isolated directly from whole peripheral blood using QIA amp DNA Blood Mini Kit (QIAGEN, Hilden Germany), and quantified with a Qubit® 3.0 Fluorimeter (Thermo Fisher Scientific) and Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). All seven exons and flanking intron regions of the *GLA* gene (GenBank: NM_000169.3) were individually PCR amplified using PCR System (Eppendorf, Master cycler Nexus, Hamburg, Germany). Primers were designed in the way that an average length of fragments was between 400 and 700 bp and that all known single nucleotide polymorphisms were avoided. We used different online available tools: single nucleotide polymorphisms Check [http://www.ngrl.org.uk], OligoAnalyzer [http://www.idtdna.com/pages/tools/oligoanalyze r], In silico PCR [http://genome.ucsc.edu]. A list of all primers is given in Table 1.

Reactions were carried out in a final volume of 25 μl containing 100 ng of genomic DNA, 1 × PCR buffer (with Mg^{2+}), 1 × Q solution, 1.0 mM $MgCl_2$, 0.2 mM dNTP each, 1 U of HotStarTaq DNA® Polymerase (QIAGEN, Hilden, Germany), 10 pmol of each primer and distilled water. Cycling conditions for PCR were as follows: initial denaturation at 95°C for 15 minutes; 30 cycles at 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for one minute, followed by a final extension at 72°C for five minutes.

All PCR fragments were directly sequenced using the Big Dye terminator cycle sequencing kit and the ABI PRISM 310 automated sequencer (Applied Biosystems Life Technologies, USA). Segregation analysis was performed in families when it was possible to determine the carrier status of detected genetic variants.

Table 1. List of all primers used for amplification of coding and flanking regions of alpha-galactosidase A (GLA) gene

Primer name	Sequence 5'-3'	Length (bp)
GLA_ex1_F	5`-AGCGGAACGTCTTACGTGAC-3`	522
GLA_ex1_R	5`-CGGGACAGTTTGCTGGG-3`	
GLA_ex2_F	5`-AGGGCGGGAATATTAACGGG-3`	620
GLA_ex2_R	5`-GTTACAGGCGTTCACCACC-3`	
GLA_ex3_F	5`-CTAGCTCAGCAGAACTGGGG-3`	522
GLA_ex3_R	5`-GAGATGGGAGCTCTGGCAC-3`	
GLA_ex4_F	5`-GGGAAGCTGAGACAGAAGAGTC-3`	477
GLA_ex4_R	5`-CCAGGTGATGGTAGCTTAGGC-3`	
GLA_ex5_F	5`-GGTTTAGACCTCCTTATGGAGACG-3`	420
GLA_ex5_R	5`-GCATCCTGCTCTAAGTACTCTCAC-3`	
GLA_ex6_F	5`-GTGAGAGTACTTAGAGCAGGATGC-3`	555
GLA_ex6_R	5`-AGCAACTAGTGATAAGTGGCCC-3`	
GLA_ex7_F	5`-CAATACCAACTTTGTCTTGGGCC-3`	675
GLA_ex7_R	5`-AGGCGGGTCTCAAAGTCC-3`	

Table 2. Patients involved in Fabry disease screening per hemodialysis (HD) center

HD center	Patients on HD (n)	Tested female patients (n)	Tested male patients (n)	Tested – both genders (n)	Age of patients
Bačka Palanka	64	4	18	22	**55.4 ± 6.4**
Stara Pazova	58	3	2	5	**36.2 ± 7.5**
Kikinda	82	5	3	8	**45.4 ± 7.1**
Subotica	70	5	9	14	**51.2 ± 6.3**
Sremska Mitrovica	120	13	29	42	**55.7 ± 4.4**
Pančevo	135	10	16	26	**47.7 ± 7.5**
In total	529	40	77	117	**48.6 ± 6.3**

RESULTS

Hemodialysis, together with peritoneal dialysis and kidney transplantation, represents methods of renal replacement therapy. There are 16 hemodialysis centers in Vojvodina, with around 1600 patients. From the yearly reports of the Registry of Serbian Society of Nephrology, we have learned that around 8% of patients with ESRD in Serbia have chronic kidney disease of unknown etiology [12].

FD is a rare disease that physicians usually don't think about in their everyday clinical practice. Having in mind that hemodialysis patients belong to a high-risk population group for FD, we decided to perform a pilot study of screening for FD patients in hemodialysis centers in Vojvodina. According to the previously mentioned criteria for screening eligibility, we have screened 117 patients (40 females) from the aforementioned hemodialysis units with a total dialysis population of 529 (22%) (Table 2). The majority of screened patients had LVH (63.2%), but stroke before the age of 55 and white matter lesions were rare (4.3% and 2.6%, respectively). Around one-fifth of screened patients also had tinnitus, hearing loss, heat or cold intolerance, postprandial pain and acroparesthesia. Complete list of comorbidities is given in Table 3.

In this study, we conducted the genetic analysis of 117 patients suspected of FD, and the *GLA* sequencing revealed only one male patient (with a frequency of 0.8%) with a variant in a coding region (p.Asp313Tyr), 16 patients (13.7%) had only non-coding variants, while 100 subjects had no variants in the analyzed regions (85.5%). All detected genetic changes were previously described in the literature. Five different combinations of number of cited variants, described as complex intronic haplotypes (CIHs), in the *GLA* gene were identified in our group of subjects (Table 4 and 5). The most frequent haplotype is formed by the four variants c.-10C>T, c.370-81_370-77delCAGCC, c.640-16A>G, c.1000-22C>T, and was detected in eight of 117 (6.8%) patients. Lyso-Gb3 biomarker levels were within normal range in each tested patient, so all genetic changes we have found could be accounted for nonpathogenic.

DISCUSSION

FD is a rare systemic metabolic disorder that leads to the accumulation of lipid substrates in lysosomes in various tissues and organs. Having in mind a fact that most important target organs in FD patients are kidneys, heart

Table 3. Comorbidities in patients involved in Fabry disease screening

Comorbidities	Bačka Palanka (N = 22)	Stara Pazova (N = 5)	Kikinda (N = 8)	Subotica (N = 14)	Sremska Mitrovica (N = 42)	Pančevo (N = 26)	In total (N = 117)
Left ventricular hypertrophy	12 (54.5%)	2 (40%)	3 (37.5%)	6 (42.8%)	28 (66.7%)	25 (96%)	74 (63.2%)
Cerebrovascular incidents < 55 years	2 (9%)	0	1 (12.5%)	1 (7.2%)	1 (2.4%)	0	5 (4.3%)
White matter lesions	1 (4.5%)	0	1 (12.5%)	0	0	1 (4%)	3 (2.6%)
Cornea verticillata	0	1 (20%)	0	0	1 (2.4%)	1 (4%)	3 (2.6%)
Angiokeratoma	0	0	0	2 (14.4%)	1 (2.4%)	0	3 (2.6%)
Tinnitus	3 (13.5%)	1 (20%)	1 (12.5%)	9 (64.3%)	2 (4.8%)	7 (27%)	23 (19.7%)
Hearing loss	8 (36.4%)	0	1 (12.5%)	2 (14.4%)	6 (14%)	4 (15%)	21 (18%)
Heat/cold intolerance	1 (4.5%)		2 (25%)	8 (57.2%)	1 (2.4%)	8 (31%)	20 (17%)
Acroparesthesia	0	2 (40%)	2 (25%)	2 (14.4%)	6 (14.3%)	13 (50%)	25 (21.4%)
Hypo-/hyperhidrosis	0	1 (20%)	0	3 (21.4%)	7 (16.4%)	0	11 (9.4%)
Post prandial pain	2 (9%)	3 (60%)	1 (12.5%)	2 (14.4%)	3 (7%)	16 (62%)	27 (23%)

Table 4. The summary of complex intron haplotypes found in patients with suspicion of Fabry disease

Genes	Alpha-galactosidase A region	Variant/Haplotype	Patients	
			N = 117	%
Variant 1	5′UTR	c.-10C>T	2	1.7
Complex intronic haplotype 1	5′UTR, intron 2, intron 4, intron 6	c.-10C>T, c.370-77_-81delCAGCC, c.640-16A>G, c.1000-22C>T	8	6.8
Complex intronic haplotype 2	intron 2, intron 4, intron 6	c.370-77_370-81delCAGCC, c.640-16A>G, c.1000-22C>T	4	3.4
Complex intronic haplotype 3	5′UTR, intron4	c.-12G>A, 639+68A>G	1	0.8
Complex intronic haplotype 4	5′UTR, intron 2, intron 4, intron 6	c.-12G>A, c.370-77_-81delCAGCC, c.640-16A>G, c.1000-22C>T	1	0.8

Table 5. Genetic changes in alpha-galactosidase A gene in our patients

Patient	Gender	Age	Genetic change	Importance
1.	♀	44	Incomplete CIH (c.-10C>T (g.1170 C>T))	Polymorphism
2.	♂	51	p.Asp313Tyr (D313Y)	Benign
3.	♀	61	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
4.	♀	32	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
5.	♀	52	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
6.	♀	47	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
7.	♀	44	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
8.	♀	53	Incomplete CIH (c.-10C>T (g.1170 C>T))	Polymorphism
9.	♂	51	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
10.	♂	46	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
11.	♀	35	Incomplete CIH (c.-12G>A (g.1168 G>A), c.639+68A>G (g.8479>G))	Polymorphism
12.	♀	63	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
13.	♂	44	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
14.	♂	29	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
15.	♂	33	CIH (c.-12 G>A (g.1168 G>A), c.639+68A>G (g.8479>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
16.	♀	66	Incomplete CIH (c.370-81_-77delCAGCC (g.7188-7192del5), c.1000-22C>T (g.10956C>T))	Polymorphism
17.	♀	53	Incomplete CIH (c.-12G>A (g.1168 G>A), c.370-81_-77delCAGCC (g.7188-7192del5), c.639+68A>G (g.8479>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism

and brain, most of the screening programs worldwide are directed at patient populations on renal replacement therapies, patients with LVH on unknown origin and patients with early cerebrovascular incidents.

In this paper we have summarized the efforts from our pilot study in the screening of hemodialysis patients from six hemodialysis centers in Vojvodina. In different screening studies on patients in hemodialysis units the prevalence of *GLA* mutations was up to 0.87% [13, 14], but if only pathogenic mutations were checked for, then the prevalence of FD dropped to around 0.14–0.3% [15]. Some screening programs are oriented just on male patients which is unacceptable, since female patients can be as severely affected as their male counterparts. Inconsistency of genotype–phenotype correlation in females is due to X-chromosome inactivation.

Detection of genetic variants in the *GLA* gene is essential to confirm the clinical diagnosis of FD [16]. In the present study, we sequenced the entire *GLA* gene including coding regions and flanking intron sequences to find alterations that could explain observed FD-like characteristics in our group of subjects. In 13.7% (16 of 117) of subjects, we identified five different combinations of CIHs in the *GLA* gene. The most frequent (6.8%) was the haplotype consisting of four variants (c.-10C>T, c.370-81_370- 77del-CAGCC, c.640-16A>G, c.1000-22C>T), which coincides with the results of other studies, where the frequency of this haplotype varied 8.9–3.4% in subjects with clinically suspected FD [17, 18].

According to van der Tol et al. [19], the prevalence of pathogenic variants in the *GLA* gene is 0.12%. When variants of uncertain significance (VUS) are included, this number increases to 0.62%. The frequencies reported in our study do not reflect the Van der Tol data since these intronic variants have been reported as polymorphic variants in the general population and, as in most cases, have been observed individually rather than in haplotypes. Furthermore, most studies perform sequencing only

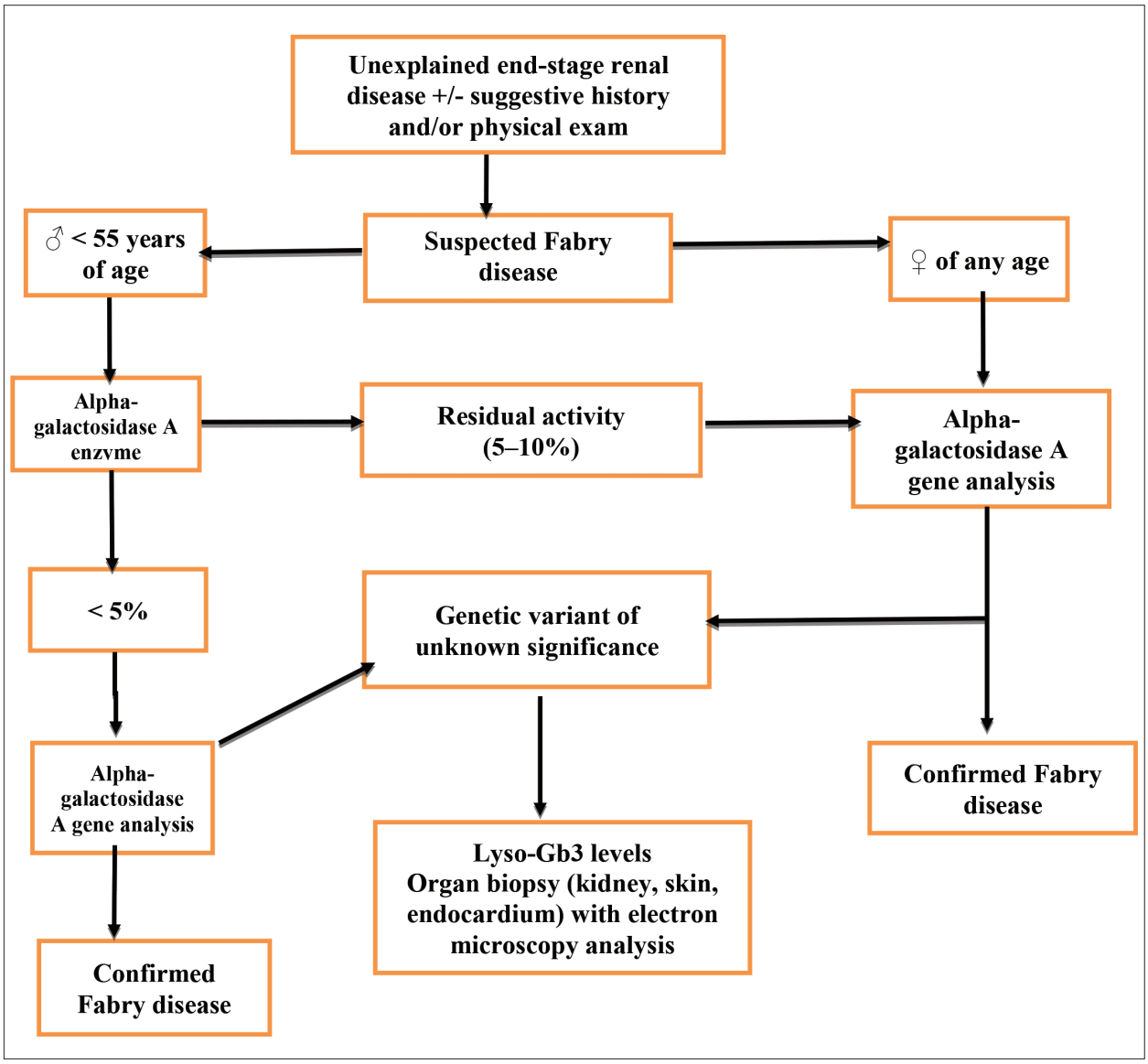

Figure 1. Diagnostic algorithm for Fabry disease patients with end-stage renal disease

through coding regions of the *GLA* gene and thus fail to detect heterozygosity in intron regions [20]. Depending on the sequencing design, CIHs often remain unidentified since these regions are not investigated routinely by gene sequencing; consequently, the prevalence of FD may be underestimated [21]. Previous reports have found that CIHs can be associated with different clinical manifestations reflecting mild renal, neurological, and cardiac disorders [22–25]. However, measuring the level of biomarker Lyso-Gb3 in DBS showed the normal level in all tested patients with detected CIHs in this study. Our results are in agreement with the findings of Ferri et al. [18], in which are detected seven different *GLA* haplotypes in control males, indicating that these CIHs are not involved in the development of FD manifestations. But Gervas-Arruga et al. [21] suggest that in patients with CIHs, environmental factors, as a pro-inflammatory state, in addition to the accumulation of Gb3 may influence the symptoms. Moreover, it has been shown that DBS lyso-Gb3 levels are not solely enough for defining diagnosis of FD and that secondary analysis should be made, due to possible false-positive or false-negative results. The estimated sensitivity of the HPLC-MS/MS method used for measuring DBS lyso-Gb3 levels is 67%, which indicates the limits of accurate/precise screening outcomes [26]. Hence, the lack of abnormality in Lyso-Gb3 levels is not a reliable parameter for excluding FD. Therefore, further analysis, such as gene expression analysis, should be performed to confirm or reject the FD diagnosis in subjects with detected CIHs in the *GLA* gene and doubtful diagnosis of disease.

High-risk population screening program represents the best and the easiest way of finding a new FD patient. A diagnostic algorithm can be proposed for screening of patients with ESRD in hemodialysis centers (Figure 1). It is based on the appropriate usage of enzyme activity testing, genetic testing as well as other types of testing that can helped physicians in determining the significance of findings during screening process. This algorithm can also

be used for patients with milder degrees of chronic kidney disease. In male patients, first step should be enzyme testing, and if needed (when enzyme levels are below normal values), further genetic testing should ensue, while in suspected female FD patients genetic testing should be the first step due to the well-known process of X-chromosome inactivation.

After appropriate high risk population screening is performed, on every index case one could find 3–5 new cases during family screening that should comprise three generations in the family tree.

Having in mind the fact that the prevalence of a pathogenic mutation in the *GLA* gene is very low in hemodialysis population, there is a place for future investigations since the total number of patients on hemodialysis in Vojvodina is around 1600 and in Serbia around 5500–6000 patients [12].

CONCLUSION

The challenges in the establishment of the precise diagnosis of FD and indications for treatment are part of today's clinical practice. In the literature has been described that the enzymatic assay is diagnostic for male patients, while female patients most often need molecular-genetic analysis to get a definite diagnosis. Moreover, the correlation between DBS enzyme activity and *GLA* variants revealed that this screening method is useful for diagnosing previously described mutations. However, when the patient presents CIH, although our study indicates a possible non-pathogenicity, the diagnosis may not be conclusive and other tools may be necessary to confirm or discard the disease. Because the genotype–phenotype correlation often shows inconsistency with FD manifestations and the effects of multiple intronic variants are not yet fully understood and seem to have individual variations, future expressional and functional studies or research focused on the discovery of possible modifier genes are necessary to confirm FD in these patients.

ACKNOWLEDGEMENTS

Genetic and biomarker analysis are sponsored by Takeda, Serbia.

Conflict of interest: None declared.

REFERENCES

1. Ortiz A, Germain DP, Desnick RJ, Politei J, Mauer M, Burlina A, et al. Fabry disease revisited: Management and treatment recommendations for adult patients. Mol Genet Metab. 2018;123(4):416–27.
2. Carnicer-Cáceres C, Arranz-Amo JA, Cea-Arestin C, Camprodon-Gomez M, Moreno-Martinez D, Lucas-Del-Pozo S, et al. Biomarkers in Fabry Disease. Implications for Clinical Diagnosis and Follow-up. J Clin Med. 2021;10(8):1664.
3. Nowak A, Beuschlein F, Sivasubramaniam V, Kasper D, Warnock DG. Lyso-Gb3 associates with adverse long-term outcome in patients with Fabry disease. J Med Genet. 2022;59(3):287–93.
4. Maruyama H, Miyata K, Mikame M, Taguchi A, Guili C, Shimura M, et al. Effectiveness of plasma lyso-Gb3 as a biomarker for selecting high-risk patients with Fabry disease from multispecialty clinics for genetic analysis. Gen Med. 2019;21(1):44–52.
5. Stenson PD, Mort M, Ball EV, Chapman M, Evans K, Azevedo L, et al. The Human Gene Mutation Database (HGMD): optimizing its use in a clinical diagnostic or research setting. Hum Genet. 2020;139(10):1197–207.
6. Oliveira JP, Ferreira S. Multiple phenotypic domains of Fabry disease and their relevance for establishing genotype – phenotype correlations. Appl Clin Genet. 2019;12:35–50.
7. Vardarli I, Rischpler C, Herrmann K, Weidemann F. Diagnosis and screening of patients with Fabry disease. Ther Clin Risk Manag. 2020;16:55–8.
8. Viggiano E, Politano L. X Chromosome Inactivation in Carriers of Fabry Disease: Review and Meta-Analysis. Int J Mol Sci. 2021;22(14):7663.
9. Germain DP, Oliveira JP, Bichet DG, Yoo HW, Hopkin RJ, Lemay R, et al. Use of a rare disease registry for establishing phenotypic classification of previously unassigned GLA variants: a consensus classification system by a multispecialty Fabry disease genotype-phenotype workgroup. J Med Genet. 2020;57(8):542–51.
10. Biegstraaten M, Arngrimsson R, Barbey F, Boks L, Cecchi F, Deegan PB, et al. Recommendations for initiation and cessation of enzyme replacement therapy in patients with Fabry disease: the European Fabry Working Group consensus document. Orphanet J Rare Dis. 2015;10:36.
11. Müntze J, Gensler D, Maniuc O, Liu D, Cairns T, Oder D, et al. Oral chaperone therapy Migalastat for treating Fabry disease: Enzymatic response and serum biomarker changes after 1 year. Clin Pharmacol Ther. 2019;105(5):1224–33.
12. Annual Report 2015. July 2017-ERA-EDTA Registry. Available on: https://www.era-edta-reg.org/files/annual reports/pdf/Ann Rep 2016.pdf
13. Jahan S, Sarathchandran S, Akhter S, Goldblatt J, Stark S, Crawford D, et al. Prevalence of Fabry disease in dialysis patients: Western Australia Fabry disease screening study – the FoRWARD study. Orphanet J Rare Dis. 2020;15(1):10.
14. Nagata A, Nasu M, Kaida Y, Nakayama Y, Kurokawa Y, Nakamura N, et al. Screening of Fabry disease in patients with chronic kidney disease in Japan. Nephrol Dial Transplant. 2021;37(1):115–25.
15. Capuano I, Garofalo C, Buonanno P, Pinelli M, Di Risi T, Feriozzi S, et al. Identifying Fabry patients in dialysis population: prevalence of GLA mutations by renal clinic screening, 1995-2019. J Nephrol. 2020;33(3):569–81.
16. Vaz-Drago R, Custodio N, Carmo-Fonseca M. Deep intronic mutation and human disease. Hum Genet. 2017;136(9):1093–111.
17. Varela P, Mastroianni Kirsztajn G, Motta FL, Martin RP, Turaca LT, Ferrer HLF, et al. Correlation between GLA variants and alpha-Galactosidase A profile in dried blood spot: an observational study in Brazilian patients. Orphanet J Rare Dis. 2020;15(1):30.
18. Ferri L, Guido C, la Marca G, Malvagia S, Cavicchi C, Fiumara A, et al. Fabry disease: polymorphic haplotypes and a novel missense mutation in the GLA gene. Clin Genet. 2012;81(3):224–33.
19. van der Tol L, Smid BE, Poorthuis BJHM, Biegstraaten M, Deprez RHL, Linthorst Ge, et al. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. J Med Genet. 2014;51(1):1–9.
20. Duro G, Zizzo C, Cammarata G, Burlina A, Burlina A, Polo G, et al. Mutations in the GLA Gene and LysoGb3: Is It Really Anderson-Fabry Disease? Int J Mol Sci. 2018; 19(12):3726.
21. Gervas-Arruga J, Cebolla JJ, Irun P, Perez-Lopez J, Plaza L, Roche JC, et al. Increased glycolipid storage produced by the inheritance of a complex intronic haplotype in the α-galactosidase A (GLA) gene. BMC Genet. 2015;16:109.
22. Tanislav C, Kaps M, Rolfs A, Bottcher T, Lackner K, Paschke E, et al. Frequency of Fabry disease in patients with small-fibre neuropathy of unknown etiology: a pilot study. Eur J Neurol. 2011;18(4):631–6.

23. Schiffmann R, Swift C, McNeill N, Benjamin ER, Castelli JP, Barth J, et al. Low frequency of Fabry disease in patients with common heart disease. Genet Med. 2018;20(7):754–9.
24. Vieitez I, Souto-Rodriguez O, Fernandez-Mosquera L, San Millan B, Teijeira S, Fernandez-Martin J ,et al. Fabry disease in the Spanish population: observational study with detection of 77 patients. Orphanet J Rare Dis. 2018;13(1):52.
25. Nakagawa N, Sawada J, Sakamoto N, Takeuchi T, Takahashi F, Maruyama J, et al. High-risk screening for Anderson-Fabry disease in patients with cardiac, renal, or neurological manifestations. J Hum Genet. 2019;64(9):891–8.
26. Linthorst GE, Vedder AC, Aerts JM, Hollak CE. Screening for Fabry disease using whole blood spots fails to identify one-third of female carriers. Clin Chim Acta. 2005;353(1–2):201–3.

Скрининг високоризичне популације на Фабријеву болест међу хемодијализним болесницима у Војводини

Дејан Ћелић[1,2], Соња Павловић[3], Анита Скакић[3], Јелица Вученовић[4], Драгана Пилиповић[5], Соња Голубовић[1,2], Татјана Стојшић[6], Оливера Милићевић[7], Горан Војиновић[8], Светлана Дудић[9], Лада Петровић[1,2], Игор Митић[1,2]

[1]Клинички центар Војводине, Клиника за нефрологију и клиничку имунологију, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Универзитет у Београду, Институт за молекуларну генетику и генетичко инжењерство, Београд, Србија;
[4]Општа болница Сремска Митровица, Центар за хемодијализу, Сремска Митровица, Србија;
[5]Дом здравља „Др Младен Стојановић", Центар за хемодијализу, Бачка Паланка, Србија;
[6]Општа болница Суботица, Центар за хемодијализу, Суботица, Србија;
[7]Општа болница Кикинда, Центар за хемодијализу, Кикинда, Србија;
[8]Општа болница Панчево, Центар за хемодијализу, Панчево, Србија;
[9]Дом здравља „Јован Јовановић Змај", Центар за хемодијализу, Стара Пазова, Србија

САЖЕТАК

Увод/Циљ Фабријева болест (ФБ) *X*-везана је лизозомна болест складиштења која се развија као последица мутације гена алфа-галактозидазе А (ГЛА). Постоји више од 1080 познатих варијанти гена ГЛА. Неки од њих су патогени, али већина их је бенигна или представљају генетску промену која се може класификовати као генетска варијанта непознатог значаја или једноставно представљати генетски полиморфизам. Постоје две главне карактеристике ФБ, класични облик и касније варијанте болести.

Главни циљни органи код болесника са овом болешћу су бубрези, срце и нервни систем. Имајући у виду чињеницу да је ФБ ретка болест, најбољи начин за активну претрагу болесника је скрининг популације високог ризика, након чега би требало извршити породични скрининг за сваки случај пробанда.

Методе У овом раду представљамо резултате мултицентричне пилот студије која представља налазе скрининга болесника на хемодијализи на ФБ у шест хемодијализних центара у Војводини.

Резултати Идентификован је један болесник са бенигном мутацијом и 16 болесника са генетским полиморфизмом гена ГЛА. Утврдили смо да генетске промене на гену ГЛА могу бити честе, али су веома ретко од клиничког значаја и ретко доводе до манифестација ФБ.

Закључак Резултати ове скрининг студије ће нам омогућити увид у преваленцију ФБ у хемодијализној популацији и усмерити наш будући рад.

Кључне речи: Фабријева болест; хемодијализа; скрининг популације високог ризика