

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

SUMMARY OF THE DOCTORAL THESIS

CARDIAC INVOLVEMENT IN FABRY DISEASE
PATIENTS IN ROMANIA

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GENERAL PART

INTRODUCTION

Fabry disease (FD) is a rare genetic disorder caused by mutations in the GLA gene on chromosome X. These mutations cause a deficiency in the secretion of a lysosomal enzyme, called alpha-galactosidase (aGal, responsible for the metabolism of globotriacylglyceride (Gb3). Therefore, Gb3 accumulation occurs in most cells, tissues and organs, resulting in a multisystem damage (1) .

I noticed a problem of underdiagnosis both globally, but especially in Romania, where, at the beginning of this study, only 14 patients with FD were diagnosed in 2015. In order to have a correct perspective on this figure, it is worth mentioning that the annual incidence of FD is estimated to be at least 1:100000 (2), which would mean that there should be at least 200 FD patients in our country.

LYSOSOMAL STORAGE DISEASES

Lysosomal storage diseases (LSD) may be caused by defects in the hydrolytic lysosomal enzymes, faulty activation of lysosomal enzymes, a defect in the intracellular trafficking of lysosomal membrane proteins. LSDs are genetic disorders and are usually autosomal recessive, with the exception of Fabry disease, Hunter disease and Danon disease (3) .

GENETIC AND MOLECULAR MECHANISMS

The GLA gene is located at the long arm of chromosome X, the exact location being Xq22.1. FD is transmitted in an X- linked manner . This means that an affected father will transmit the gene responsible for the disease to all his daughters and to no boy, while an affected mother will transmit the gene with a 50% chance to any child regardless of gender .

EXTRACARDIAC INVOLVEMENT

Skin lesions, called angiokeratoma are the most familiar feature of the FD even today. They are red and purple maculopapular lesions that occur mainly in the groin, umbilical, lip, palmar and plantar areas (especially at the distal end of the fingers). They are generally small in size (<5 mm) and are rarely solitary or may fuse and form placards. Their formation occurs by small vessel dilation that become dysfunctional, leading to the characteristic appearance visible in the epidermis. Angiokeratomas are generally asymptomatic, being partially evacuated under pressure, but in some cases they can lead to bleeding especially if they are present in the genital mucosa (4) .

Along with the cardiac and central nervous system, renal impairment in FD is implicated in the vital prognosis of patients. Renal impairment is characterized by proteinuria and the progressive decrease in renal function, and is due to the accumulation of Gb3 with predilection in the glomeruli (especially in the podocytes) and in the distal convoluted tubule. These locations also explain that in many cases proteinuria is the first manifestation of kidney damage in Fabry disease.

Peripheral neuropathy in FD is characterized by the presence of distal limb pain, decreased tolerance to extreme temperatures and gastrointestinal disturbances (5). Another feature associated with peripheral neuropathy in FD is dysfunction of exocrine sweat glands.

Central nervous system involvement is not an involvement of the nervous tissue, but is secondary to cerebral vascular damage and may be manifested through ischemic stroke, generally in young adults.

FD ocular damage is common and is manifested by corneal opacities (cornea verticillata) and retinal or conjunctival vascular abnormalities. From the vascular point of view, the sign described is accentuated tortuosity.

Auditory impairment is relatively common in FD. The manifestations may vary from tinnitus to hearing loss. The progression of hearing loss was described as similar to that of the general population, but with early onset, however, in some cases it may occur suddenly (6) .

CARDIAC INVOLVEMENT

Involvement of the heart is a major cause of morbi-mortality in Fabry disease. In general, Fabry's cardiomyopathy is described as a form of non-obstructive hypertrophic cardiomyopathy, characterized by left ventricular concentric

hypertrophy. A sphingolipid cumulation occurs in both the myocytes, the cells of the electric system, smooth muscle cells, vascular endothelium or fibroblasts (7) .

From a cardiological point of view, in FD, there are no characteristic signs and symptoms.

Troponin can play an important role in detecting FD. Elevated levels in Fabry cardiomyopathy have been documented in numerous case studies and reports (8-10) .

Electrophysiologically, there is an early shortening of the PR interval (11), change that often persists even after the development of hypertrophy. Then, with disease progression, there are signs of hypertrophy and conduction (atrioventricular block) or rhythm disorders.

The first objective of echocardiography in FD is to identify the presence of ventricular hypertrophy . Although in most cases, left ventricular hypertrophy is concentric, non-obstructive, sometimes obstructive or severe asymmetric forms can be encountered in patients with FD (12) . Rarely, a hyperechogenic area can be visualized in the inferolateral portion of the left ventricular myocardium in the parasternal views. Several studies have shown a significant presence of right ventricular hypertrophy in Fabry cardiomyopathy (13,14). One of the signs described as being specific in Fabry's cardiomyopathy to other forms of LVH is hypertrophy of papillary muscles (15). Global longitudinal strain is altered in FD patients who have myocardial fibrosis.

Cardiac magnetic resonance is not only more accurate than echocardiography in measuring the heart chambers and the thickness of their walls, but it also offers the possibility to identify and characterize myocardial tissue pathological processes such as inflammation or fibrosis. Myocardial edema may be assessed visually by observing the hypersignal on T2-weighted images or by calculating the mean T2 on T2 mapping images. Myocardial fibrosis highlighted as intra myocardial hypersignal can be detected using late enhancement sequences. Multiple studies have reported low values of native T1 in patients with FD. By accumulation of lipid material in the myocardium, T1 decreases, which is a characteristic of FD and cardiac iron loading. In FD, the characteristic location of fibrosis is the inferolateral area of LV with a pattern that does not correspond to a coronary territory and is generally intramural. However, almost a quarter of FD patients have fibrosis with atypical location either associated with the inferolateral one or associated with asymmetric hypertrophy (16). Significant

fibrosis may be a predictor of the occurrence of arrhythmic events and enzymatic treatment unresponsiveness (17-18).

POSITIVE DIAGNOSIS

The positive diagnosis of FD is determined based on the biological assay of the AGAL enzyme level and genetic analysis of the GLA gene. For men, it is sufficient to detect a low enzyme level to establish FD diagnosis, whereas for women, it is necessary to find a specific genetic mutation, due to the fact that women may have a residual production of AGAL.

TREATMENT

General treatment in FD is the treatment of complications and organ involvement. Therefore, in patients with symptoms of heart failure, it is recommended to treat with enzyme inhibitors or angiotensin receptor blockers conversion.

Specific treatment in FD is intended to treat the cause of the disease, i.e. to increase the lysosomal enzymatic activity of AGAL and to reduce sphingolipid deposits. For this purpose, first developed and most commonly used therapy is enzymatic therapy. In Romania, enzyme therapy is administered free of charge to patients with FD, based on a medical record, through the National Program for Rare Diseases.

OWN CONTRIBUTIONS

INTRODUCTION, MOTIVATION AND OBJECTIVES

The motivation for this study came from the need to analyze Fabry disease in Romania, where, prior to the start of this study, there was no centralization of these patients' data. FD is severely underdiagnosed in our country compared to the prevalence data demonstrated by many studies.

MATERIAL AND METHODS

All patients in Romania with a positive genetic diagnosis for FD were included in the study by 2018 comprising a total of 42 patients. For each patient,

123 anamnestic, clinical, biological and imaging parameters (including echocardiographic deformation analysis parameters and CMR parameters) were entered into the database .

DESCRIPTIVE RESULTS

The mean age of the patients enrolled in the study was 47 ± 15 years. Most patients were in the age group of 40-60 years (55%), while only 13 of the patients were under 40 and 7 over 60 years of age. The extremes were 19 years and 80 years respectively. Distribution by gender was relatively equal: 19 men (45%) and 23 women (55%). Women were on average 12 years older (52 ± 15 years vs 40 ± 13 years). The study included 20 families, representing an average of 2-3 members diagnosed with FD per family .

Of extracardiac impairment, the most common was neurological involvement, 52% of patients. Skin manifestations were present in 48% of patients, represented by the presence of angiokeratomas. Renal impairment, defined as significant proteinuria or $\text{GFR} < 60 \text{ ml / min / } 1.73 \text{ m}^2$, was present in 39% of patients. Only a small proportion of patients (16%) had normal renal function according to the classification. Five patients had a severe impairment of renal function (stage IV and V), and two of them were in stage V of BRC, being on dialysis. Cornea verticillata as a sign of ocular involvement was present at 35% and ENT manifestations in 30% of patients.

Some patients declared cardiac symptomatology. Thus, 38% reported fatigue and 26% palpitations. A relatively low number experienced angina (6 patients), lipothymia (4 patients) or syncope (3 patients).

36% had an elevated BNP and the same amount of patients had elevated troponin levels.

33% of patients had a short PR interval; none of them having a Delta wave. 7 patients had a pace-maker: 5 women and 2 men.

When analyzing the echocardiographic parameters some pathological changes were observed. Thus, 20 patients (48%) showed LVH according to cut-off of maximum wall thickness $> 12 \text{ mm}$. All the same patients (48%) exceeded the limit of their normal values for the cut-off of LVH according to the ventricular mass indexed to body surface area: $> 115 \text{ g/m}^2$ for men and 95 g/m^2 for women.

The LVEF was normal in most patients, with only 3 patients having LVEF <55% and one patient <45% (mean LVEF was 63%)

In the study group, the average area of the papillary muscles was 2.8 cm², and 7 patients had a higher value than the cut-off value mentioned. The visual assessment of papillary muscle hypertrophy has been shown to have a good sensitivity and specificity by the same authors. Thus, in our group, papillary hypertrophy evaluated visually was present in 15 patients (36%). 48% of patients had septal e' <8 cm/s, 43% had septal S ≤ 8 cm/s and 17% had an E/e' ratio > 14. It is worth noting the GLS average was below the normal limit (-16%) in our group. Of the study group, 58% had GLS outside the normal range.

In CMR, almost half (47%) of patients had a maximum left ventricular wall thickness of > 12 mm. Based on the visual analysis of late contrast images, LGE was identified in 4 patients (21%).

ECOCARDIOGRAPHIC PARAMETERS THAT DISTINGUISH FABRY CARDIOMYOPATHY FROM SARCOMERIC HYPERTROPHIC CARDIOMIOPATHY

Through this sub-study I tried to identify the echocardiographic parameters that differentiate Fabry's cardiomyopathy from sarcomeric hypertrophic cardiomyopathy and two groups of patients were selected (20 patients with FD and LVH and 20 patients with sarcomeric HCM).

Parameters that significantly differentiated the two groups were as follows

Variable	All (40)	Fabry (20)	HCM (20)	p
LVWA	1.39 ± .66	1.22 ± .63	1.55 ± .66	.001
LVWA > 1.3	13 (33%)	3 (15%)	10 (50%)	.018
ESDLV (mm)	25 ± 7	28 ± 7	22 ± 5	.004
LVEF (%)	68 ± 8	63 ± 7	72 ± 7	.001
LVOT obstruction	12 (30%)	3 (15%)	9 (45%)	.038
Mitral SAM	13 (33%)	3 (15%)	10 (50%)	.018
IL hyperechogenic area	4 (10%)	4 (20%)	0 (0%)	.035
Inferolateral LV LS (%)	-12 ± 7	-9 ± 5	-16 ± 7	.006
RV free wall LS (%)	-26 ± 6	-23 ± 6	-28 ± 5	.027

CORRELATION OF ECOGRAPHIC PARAMETERS WITH THE PRESENCE OF MYOCARDIAL FIBROSIS ON CARDIAC MAGNETIC RESONANCE

For this sub study, I aimed to find echocardiographic parameters that can predict the presence of myocardial fibrosis. CMR was available in 19 patients with FD. 4 of the patients had myocardial fibrosis.

The parameters that predicted significantly the presence of fibrosis were as follows:

Parameter	No fibrosis (15)	Fibrosis (4)	p
MWT (mm)	11 ± 2	20 ± 8	> .001
LV mass i (g/m²)	87 ± 18	226 ± 144	.001
PapM (cm²)	2.09 ± 0.96	4.25 ± 1.55	.003
PapM area/LV Circumf (midLV)	0.12 ± 0.05	0.22 ± 0.08	.009
LAVi (ml / m²)	29 ± 7	49 ± 14	.001
S' septal (cm / s)	8 ± 1	6 ± 2	.002
E mitral (cm / s)	86 ± 13	70 ± 5	.029
e' septal (cm / s)	9 ± 3	4 ± 2	.002
E / e'	10 ± 3	21 ± 10	.001
GLS (%)	-18 ± 2	-14 ± 5	.010
LV IL LS (%)	-13 ± 5	-7 ± 6	.032
RV free wall (mm)	5 ± 1	8 ± 3	.027

DISCUSSIONS AND CONCLUSIONS

The main limitation is the relatively small number of patients, this being true both for the descriptive analysis and for the two subsequent sub studies. A small number of subjects leads to the predisposition to statistical errors of both type 1 (false positives) and type 2 (false negatives). However, this was the total number of patients with Fabry disease in Romania at the time of conclusion of the study.

The main conclusion of this paper is that patients with Fabry disease in Romania represent a population that has long been neglected due to the rarity of their pathology, but which have an important degree of multiorgan involvement and family burden, with a considerable psychological impact.

A supranormal ejection fraction is not characteristic of Fabry disease compared to sarcomeric HCM and inferolateral involvement is specific to Fabry

cardiomyopathy, illustrated by altered longitudinal strain or by changes in echogenicity at this level. Also, concentric hypertrophy wherein the LV posterior wall is greater than or equal to the interventricular septum could exclude sarcomeric HCM and guide diagnosis toward another etiology (such as Fabry cardiomyopathy).

Patients with myocardial fibrosis associate several altered echographic parameters, including the LV maximum wall thickness and mass, but also diastolic function and cardiac deformation parameters.

In the future, to be validated, the results of this analysis will need to be checked by larger, possibly multicenter studies, due to the rarity of the pathology.

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