Screening of Fabry Disease of patients in renal replacement therapy in a population from Lazio (Italy)

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Abstract. – OBJECTIVE: Fabry’s disease (FD) is a genetic disorder of lysosomal storage characterized by the intralysosomal accumulation of globotriaosylceramide (Gb3). This genetic mutation causes a total or partial deficit of the α-galactosidase (GAL) enzyme activity. FD has an incidence of 1:40000-60000 born alive. Its prevalence is higher in specific pathological conditions like chronic kidney disease (CKD). The aim of this study was to evaluate the FD prevalence in Italian renal replacement therapy (RRT) patients from Lazio region.

METHODS: 485 patients in RRT (hemodialysis, peritoneal dialysis, and kidney transplantation) were recruited. The screening test was performed on venous blood sample. The latter was analyzed using specific FD diagnostic kit, based on the analysis of dried blood spots on filter paper.

RESULTS: We found 3 cases of positivity to FD (1 female and 2 males). In addition, 1 male patient was identified with biochemical alteration indicative of GAL enzyme deficiency with a genetic variant of the GLA gene of unknown clinical significance. The FD prevalence in our population was 0.60% (1 case out 163), it rises to 0.80% (1 case out of 122) if the genetic variant of unknown clinical significance is considered. Comparing the three subpopulations, we observed a statistically significant difference in GAL activity in transplanted patients compared to dialysis patients (p<0.001).

CONCLUSIONS: Considering the presence of an enzyme replacement therapy able to modify FD clinical history, it is essential to try to implement FD early diagnoses. However, the screening is too expensive to be extended on large scale, due to the low prevalence of the pathology. The screening should be performed on high-risk populations.

Key Words: Fabry disease, Renal replacement therapy, Kidney transplantation, End stage renal disease, Lysosomal storage disease, Alpha-galactosidase, Lyso-Gb3.

Introduction

Fabry disease (FD) is a genetic disorder of lysosomal storage with recessive X-linked transmission, characterized by the intralysosomal accumulation of globotriaosylceramide (Gb3) due to the mutation, in chromosome Xq22.1, of the GLA gene1. This genetic mutation causes a total or partial deficit of the α-galactosidase (GAL) enzyme activity, which leads to an alteration of the metabolism of some glycosphingolipids with consequent intralysosomal accumulation of Gb3 in various tissues and organs2. This process results in a multisystem disease that mainly involves heart, kidneys, nervous system, skin and eyes3.

From an epidemiological point of view, FD is an extremely rare condition with an incidence of 1:40000-60000 born alive4. More recent studies, based on neonatal molecular screening programs, show a higher incidence, up to 1:3100 live births5. Furthermore, the prevalence appears to be higher in specific high-risk subpopulations such as young patients with cryptogenic stroke6, patients...
without other causes of hypertrophic cardiomyopathy or patients with chronic kidney disease (CKD) by unknown cause.

The disease, having an X-linked recessive transmission, presents different clinical manifestations according to the residual activity of the GAL enzyme and to the gender. Male patients with total deletion of the GLA gene present the classic clinical manifestation of the disease, while female patients may have different clinical pictures, depending on the random inactivation of the X chromosome (Lyonization). In fact, patients with the classic phenotype have mutations that cause the complete absence of GAL, while patients with missense mutation have a residual enzyme activity from 2 to 25% and, consequently, have a more “nuanced” phenotype. The last is characterized by an increased risk of developing cardiovascular diseases (CVDs) and/or, in case of CKD, to develop a rapid progression towards end-stage renal disease (ESRD).

Therefore, based on the GAL residual enzyme activity, we can recognize a classic manifestation of FD (type 1) and a late-onset variant (type 2).

Classic FD occurs in pediatric age with episodic pain in the extremity, fever of unknown origin, abdominal pain, and diarrhea after meals with a high lipid content. During the adolescence, the first pathognomonic signs of the disease, such as angiokeratomas, namely vascular skin anomalies characterized by small red or blue papules, appear. Angiokeratomas are frequently multiple and with diffuse distribution (angiokeratoma corporis diffusum). Ophthalmological manifestations are common and the main among these is represented by the vortical keratopathy with subepithelial corneal spiral accumulation (cornea verticillata). As it is universally acknowledged, the disease may progress with additional symptoms, resulting from alterations in the nervous, cardiovascular, or renal systems with an increased risk of developing complications, such as failure, the arrhythmic pathology, the hypertrophic cardiomyopathy, the ischemic stroke with possible evolution in the hemorrhagic stroke and a rapid progression towards ESRD.

On the other hand, late-onset FD is characterized by the involvement of only one organ (kidney or heart) and by the onset of pathognomonic symptoms in adulthood. In these cases, chronic insults related to FD, added to the traditional risk factors, lead to early cardiac and renal complications, and could cause misdiagnosis and subsequent failure to a therapeutic treatment. The clinical manifestations are due to the chronic intralysosomal accumulation of not completely catabolized α-D-galactose fragments such as Gb3, which seem to induce a state of chronic cellular toxicity.

Furthermore, blood values of globotriaosylsphingosine (lyso-Gb3), the deacetylated form of Gb3, are up to 50 times higher in FD patients than in healthy controls. Lyso-Gb3 is a soluble molecule that can be easily metabolized from the cells, and it is generated by the activity of the acid ceramidase, a lysosomal enzyme. Lyso-Gb3 is an inhibitor of GAL A and B enzymes and a promoter of vascular smooth muscle cells replication, and thus, it can have a key-role in increasing myo-intimal thickness. Although the action of lyso-Gb3 in the FD pathogenesis remains unclear, it is useful for monitoring the disease severity and the therapeutic response.

This study aimed to evaluate the prevalence of FD in the Lazio region (Italy) population in renal replacement therapy (RRT) and secondly to analyze the possible differences in GAL enzyme activity in different RRTs in ESRD patients without FD.

**Patients and Methods**

**Enrolled Patients**

In the present study, 485 patients in RRT (such as hemodialysis, peritoneal dialysis, or kidney transplantation) were recruited from UOC of Internal Medicine-Centre of Hypertension and Nephrology Unit and UOC of Transplantation of University Hospital Policlinico Tor Vergata (PTV) out-patients, from May 2018 to May 2020. The study protocol followed the Helsinki declaration and was approved by the University Hospital PTV Independent Ethics Committee (trial number 8466/2018, Clinical Experimentation Register of PTV). All enrolled patients signed an informed consent before starting the study. The inclusion criteria were patients in RRT, age ≥18 years old, both sex, and acceptance of informed consent. The exclusion criteria were the presence of HIV, HbsAg+ and HCV+.

At the time of the enrollment, all anamnestic data were collected for each patient. In addition, a physician provided general information about FD in each patient.

**Methods**

The screening test was performed on venous blood sample. The latter has been analyzed using specific FD diagnostic kit, based on the analysis
of dried blood spots. In particular, the latter had been left to dry on a filter paper at room temperature for two hours. The sample analysis has been performed at CENTOGENE, (CENTOGENE AG laboratories, Rostock, Germany).

The study design is illustrated in Figure 1. For male patients, the analysis firstly evaluated the GAL enzyme activity (normal values ≥15.3 µmol/L/h with immunofluorimetry) and then, for subjects with pathological enzyme activity, it was performed the genetic assessment of the GLA gene (NGS-Illumina®, CENTOGENE, Rostock, Germany) to detect known pathogenic mutations, new genetic variants, or those with unknown clinical significance.

The method used to analyze GLA gene in next generation sequencing (NGS) is based on the developing and sequencing of amplicons. Amplicons occupy all coding regions and highly conserved splicing junctions between exons and introns. This method ensures a minimum coverage of more than 20x for each amplicon. It was also performed lyso-Gb3 analysis to evaluate the disease severity (normal values between 0.08-1.13 nmol/L with liquid chromatography-MS). Female patients directly underwent the genetic test, as the enzyme activity is non-diagnostic for the X-chromosome Lyonization.

FD positive patients underwent a further specific genetic test in order to identify their own allelic variant. Moreover, we extended the FD screening to the relatives of the positive patients.

**Statistical Analysis**

All data was initially entered into an Excel spreadsheet (Microsoft, Redmond, WA, USA) and the analysis was performed using the Windows Statistical Package for Social Science, version 15.0 (SPSS Inc., Chicago, IL, USA). The descriptive statistics consider the mean ± standard deviation (SD) for the parameters with normal distribution (after confirmation with histograms and the Kolgomorov-Smirnov test), while for the non-normal variables, they consider the median and the interval (minimum-maximum).

The comparison of the data of normal variables with the non-parametric ones were carried out with a one-way ANOVA. Regarding occurrences (percentages), the chi-square test, possibly corrected by Fisher’s exact test, was performed. A p-value <0.05 was considered statistically significant.

**Results**

The epidemiological findings of the study population are illustrated in Table I. The mean age of the all-enrolled patients was 57.6±11.6 years. Of the 485 patients analyzed, 155 (31.9%) were female while 330 (68.1%) were male. One
hundred fourteen patients underwent hemodialysis, including 35 females and 79 males, 49 patients underwent peritoneal dialysis as RRT, 19 of which were female and 30 males.

The two groups of patients in dialysis (namely in hemodialysis or in peritoneal dialysis) were homogeneous for gender \((p=0.863)\) and age \((p=0.939)\). In hemodialysis group, 29 patients (25%) underwent biopsy and histological examination of the native kidneys, while in the peritoneal dialysis group, only 9 patients (18%) received this diagnostic procedure.

At the moment of the venous sample, patients with functional kidney transplant were 322 (65.8%), with an average age of 58.3±12.1, of which 101 females and 221 males. As stated before, the kidney transplanted patients presented a statistically significant gender disproportion \((p=0.007)\). One-hundred eighty-nine patients (58.7%) who underwent kidney transplantation did not perform histological examination of the native kidneys.

Among the renal transplanted patients, 233 underwent hemodialysis treatment, before the surgical procedure. Sixteen of the kidney-transplanted patients reported a medical history of type 2 diabetes mellitus, while 176 of them reported systemic arterial hypertension.

Within the 485 enrolled patients, 3 cases of positivity to FD were found (1 female and 2 males). Moreover, 1 male patient was identified with biochemical alterations indicative of GAL enzyme deficiency with a genetic variant of the GLA gene of unknown clinical significance. In detail, we observed the variant c.937G>T (p.Asp313Tyr), detected in exon 6 of the GLA gene, that causes an amino acid change from Asp to Tyr in position 313 (p.D313Y). According to a systematic review\(^27\), which analyzed 35 studies, the patients with GLA (p.D313Y) showed a low prevalence in typical FD clinical manifestations and a higher enzyme activity compared to patients with the FD classic phenotype. Indeed, according to a study conducted by Lenders et al\(^28\), this variant could represent a possible risk factor for neurological disorders, such as white matter lesions. Moreover, this variant should be evaluated in case of absence of other classic risk factors for neurological disease. This observation was confirmed in a meta-analysis conducted by Palaiodimou et al\(^29\). They highlighted that the variant p.D313Y seems to be related to milder FD clinical manifestations and to a late development of the phenotype, in which prevail the neurological disorders.

The patient with an ascertained diagnosis was treated with hemodialysis and subsequently transplanted, while 2 FD patients were treated with peritoneal dialysis, and 1 FD with hemodialysis, before the kidney transplantation.

Two of 3 FD positive patients received a previous diagnosis as cause of ESRD instead of FD, namely a probable nephroangiosclerosis and a lupus nephritis.

The prevalence of FD in our population was 0.60% (1 case out of 163), it could rise to 0.80% (1 case out of 122) if the genetic variant of unknown clinical significance was considered. The median age of patients with FD diagnosis was 49 (45-60) years.

**Discussion**

Data concerning the values of lyso-Gb3 and GAL enzyme activity of the study population, divided according to RRTs, were reported in Table II. Comparing the three subpopulations for these parameters, we observed a statistically significant difference in GAL enzyme activity in transplanted patients compared to dialysis patients \((p<0.001)\). Moreover, comparing the lyso-Gb3 values among the three subpopulations, we did not observe any statistical significance.

The FD prevalence in our study population, excluding the variant of uncertain significance, was
suppressive regimen of our cohort, can decrease the titre of anti-GAL IgG and might prevent the enzyme inhibition. This pathway could explain the increased enzyme activity levels observed in our kidney transplanted population. Furthermore, Lenders et al. showed that patients with FD, who were started enzyme replacement therapy (ERT) after transplantation, did not develop new antibodies against ERT in long-term follow up, supporting the protective effect of immunosuppression on FD. Interestingly, the efficacy of the immunosuppression to counteract the ERT inhibition, decreased when the immunosuppressive drug blood levels were downtitrated. Additionally, the optimal immunosuppressive regimen and ideal dosages against formation of anti-GAL antibodies need to be further investigated.

We speculate that uremic toxins induce a possible inhibiting action on GAL enzyme. In order to support this hypothesis, further clinical studies could be necessary to evaluate how the uremic toxins and the oxidative stress impact on GAL enzyme activity. In fact, CKD is characterized by several clinical manifestations and by numerous metabolic dysfunctions, not all well-known yet. It could be hypothesized that CKD itself impacts on various enzymatic pathways and among these, GAL enzyme activity. Currently, the only study that examined the GAL enzyme activity in kidney transplanted patients without FD supports our findings, namely this high-risk population presents lower GAL enzyme activity compared to general population.38

A limitation of our monocentric study is the low sample size. To overcome this, it is essential to conduct similar studies in order to broaden the sample and make the evidence more significant. Considering that it is a genetic pathology, it will be important to collect new data both on Italian population and on other countries’ populations, from all over the world. It is notable to detect the possible differences of FD prevalence related to ethnicity, in order to select high-risk populations, not only based on comorbidities but also on ethnicity.

Table II. Lyso-Gb3 and GAL values of the screened population, divided according to RRT.

<table>
<thead>
<tr>
<th></th>
<th>Hemodialysis</th>
<th>Peritoneal dialysis</th>
<th>Kidney Transplantation</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyso-Gb3 (ng/ml)</td>
<td>1.49±1.50</td>
<td>1.16±0.40</td>
<td>1.44±0.56</td>
<td>1.39±0.55</td>
</tr>
<tr>
<td>GAL (ng/ml/h)</td>
<td>18.28±14.36</td>
<td>18.82±14.45</td>
<td>21.33±15.16</td>
<td>18.99±16.34</td>
</tr>
</tbody>
</table>
Conclusions

Considering the presence of an ERT able to modify the clinical development of FD, it is essential to try to implement FD early diagnoses. However, the screening is too expensive to be extended on large scale, due to the low prevalence of pathology (1: 20000-40000). The screening should be performed on high-risk populations, for example on CKD patients under conservative therapy or in RRT (hemodialysis, peritoneal dialysis, kidney transplantation). In particular, this screening should be conducted on CKD patients in early stages in order to slow down the progression of CKD itself and to permit, in the case of missed diagnosis, the identification of the actual CKD primary cause.

The screening on ESRD patients has important implications both directly on their health status, and indirectly on their family. Once the ESRD has been reached, even counteracting the accumulation of FD catabolites, the renal recovery is marginal. Therefore, limiting the amount of waste products can mostly reduce either the onset of the comorbidities related to CKD or the systemic manifestations.

In addition, in FD kidney transplanted patients, ERT may slow down the accumulation of catabolites related to FD and therefore the CKD progression.

Moreover, the diagnosis of FD, as it is a genetic pathology, allows to obtain an important impact on the health of the relatives, who could thus receive an early diagnosis. Consequently, an adequate genetic counselling is important to identify any possible carrier of the mutation, who should undergo further investigations.

Conflicts of Interest
The authors declare no conflicts of interest.

Informed Consent
Informed consent was obtained from all individual participants included in the study.

Ethics Approval
The study protocol, following the Helsinki declaration, was approved by University Hospital PTV Independent Ethics Committee (trial number 8466/2018, Clinical Experimentation Register of PTV).

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References


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