Case report: is low α-Gal enzyme activity sufficient to establish the diagnosis of Fabry disease?

Relato de caso: a diminuição da enzima α-Gal é suficiente para estabelecer o diagnóstico?

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Abstract

Fabry disease is an X-linked lysosomal storage disease due to alpha-galactosidase A (α-Gal A) deficient activity which leads to the accumulation of glucoesphingolipids, such as globotriaosilceramide. There are over 700 known mutations of the enzyme gene, and most of them cause Fabry Disease. This case report describes a hemodialysis patient with a rare and controversial GLA gene mutation, the D313Y. The medecial investigation confirmed that D313Y is an alpha-galactosidase A sequence variant that causes pseudo deficient enzyme activity in plasma but not Fabry disease. Thus, clinical symptoms that prompted Fabry disease investigation could not be attributable to Fabry disease and therefore enzyme replacement therapy was not indicated.

Keywords: Fabry disease; diagnosis; genetic diseases, X-linked; alpha-galactosidase; renal dialysis; mutation.

Resumo

Doença de Fabry (DF) é uma doença de depósito lisossômico ligada ao cromossomo X, causada pela deficiência da enzima alfa-galactosidase A (α-Gal A) que leva ao acúmulo de glicoesfingolipídeos, principalmente globotriaosilceramide. Existem mais de 700 mutações conhecidas do gene da enzima, a maioria delas são causadoras de DF. Este relato de caso descreve sobre um paciente em hemodiálise com uma mutação do gene GLA rara e controversa, a D313Y. A investigação médica confirmou que D313Y é uma variante que leva à pseudodeficiência plasmática da enzima, mas não ocasiona DF. Assim, os sintomas clínicos que induziram a investigação da doença não devem ser atribuídos à DF e, portanto, não foi indicada a terapia de reposição enzimática.

Palavras-chave: doença de Fabry; diagnóstico; doenças genéticas ligadas ao cromossomo X; alfa-galactosidase; diálise renal; mutação.

Introduction

Fabry disease is an X-linked lysosomal storage disease caused by alpha-galactosidase A (GLA) gene mutation, which leads to an alfa-galactosidase A enzyme (α-Gal A) deficiency and consequently accumulation of glycosphingolipids, mainly globotriaosilceramide. Globotriaosylceramide accumulates within lysosomes throughout the body, leading to complications on different organs and systems, such as central and peripheral nervous systems, the kidneys and the cardiovascular system.[1] More than 700 mutations in the GLA gene have been recognized.[2] Importantly, GLA mutations can also be non-pathogenic.

Classical manifestations of Fabry disease begin on life's first decade, which comprise neuropathic pain, hypohidrosis, angiokeratomas, cochleovestibular and gastrointestinal disorders. Usually, after the second decade of life, transient vascular ischemia and stroke, chronic kidney disease (CKD), manifested by proteinuria and/or reduced glomerular filtration rate, cornea verticillata, cardiomyopathy and arrhythmia may appear.[1] Late-onset phenotypes with predominance of the involvement of the heart or the kidneys have also been described.[3,4]

Fabry disease has been described in several ethnic groups with an estimated prevalence of 1:40,000 to 1:170,000

males.[1] Taking into consideration that Fabry disease is rare and its clinical manifestations may be slight, particularly in the late onset subtypes, one should be aware that the true prevalence of Fabry disease has probably been underestimated. In agreement with this hypothesis, recent screening surveys in newborn reported an incidence of 1:3,100.[5] Among the high-risk population, such as individuals with idiopathic left ventricular hypertrophy or those in renal replacement therapy, the prevalence seems to be higher.[1]

In Brazil, the prevalence of Fabry disease among male hemodialysis patients ranges from 0.12% to 0.57%.[6-8] The development of cheaper diagnostic tools, such as the dried blood spot on filter paper, has facilitated the screening of high-risk populations. This strategy may allow the identification of Fabry disease patients in renal replacement therapy centers, spreading the diagnosis to relatives in earlier stages. In the other hand, nephrologists will be challenged to confirm or refuse the clinical diagnosis of Fabry disease from a positive screening result in each tested patient.

Herein, in this case report, we describe a carrier of the D313Y mutation, identified by screening of Fabry disease in hemodialysis male patients. This mutation is considered controversial in the literature. The patient had clinical findings, such as left ventricular hypertrophy, arrhythmia and end-stage renal disease (ESRD), which could be attributed to Fabry disease. This diagnosis was not confirmed, though. This case report highlights the importance of careful evaluation of suspected cases of Fabry disease, particularly when controversial mutations, new mutations or mutations of unknown significance are detected, in order to avoid misdiagnosis and inadequate indication of enzyme replacement therapy (ERT).

Case report

A 44-year-old man on hemodialysis for seven years was enrolled in a high risk population screening for Fabry disease in 2015. His α-Gal A enzyme activity in dried blood spot was 1.24 µmol/L/h (reference: > 2.2 µmol/L/h). Due to low enzymatic activity, genetic test was performed. The nonsense mutation p.D313Y in exon 6, in homozygosis, was detected.

Patient reported periorbital swelling and fatigue for 10 years and systemic hypertension for more than 20 years. He denied ischemic coronary artery disease or stroke. He had no family history of CKD. He was a current smoker (40 packs/year); and stopped alcohol consumption 4 years before. He was taking the following medications: clonidine, atenolol, calcium carbonate, and erythropoietin. He had episodes of arrhythmia during hemodialysis session and discoid lupus diagnosis for 3 years, for which he has been using corticosteroid and chloroquine.

The presence of clinical manifestations (Table 1) of Fabry disease was further investigated. The electrocardiogram revealed sinus rhythm, left ventricular hypertrophy and subepicardial ischemia of inferior and lateral walls. The echocardiogram showed a moderate concentric hypertrophy of the left ventricle with preserved dimension and function. Cornea verticillata was not detected. Magnetic resonance imaging (MRI), indicated to investigate white matter lesions, was not performed due to the risk of nephrogenic fibrosis secondary to gadolinium exposure.[9]

Table 1 Correspondence among main clinical manifestations in Fabry disease and in D131Y hemodialysis patient

Fabry disease manifestations	D313Y patient findings
Low enzymatic activity	Yes
GLA gene mutation	Yes
Acroparaesthesia	absent
Angiokeratomas	absent
Hypohidrose or hyperhidrosis	absent
Cornea verticilatta	absent
Chronic kidney disease	Yes
Left ventricular hyperthrophy	Yes
Diastolyc dysfunction	Yes
Conduction abnormalities	Yes
Cerebrovascular alteration	Not investigated
Globotriaosylceramide deposits on tissue	absent
CONCLUSION	
The diagnosis of Fabry Disease was refused based on the absence of globotriaosylceramide deposits in tissue.	

Importantly, the patient was submitted to a kidney biopsy in other nephrology service, before starting hemodialysis. The kidney tissue was stained with hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, and silver. It contained 8 glomerulae (5 sclerotic, 3 with hyalinosis of glomerular capillary). Tubules and intersticium presented signs of chronicity. Severe arteriosclerosis and arteriolosclerosis were the

most significant alterations detected in the vascular compartment. In conclusion, histopathologic findings were suggestive of hypertensive nephrosclerosis. No histologic changes of Fabry nephropathy, such as vacuolization of podocytes, were identified by light microscopy.

Regarding his family history, the patient had 2 daughters. The genetic analysis performed in one of them unveiled, as expected, the same mutation p.D313Y. The other daughter refused the genetic test. Both daughters had no clinical manifestations of Fabry disease.

Discussion

The patient presented the mutation p.D313Y, substitution of GAT for TAT in cDNA 937 nucleotide, in the GLA gene. This mutation causes a unique substitution of an aspartate for a tyrosine, in +313 position.[10] This leads to the formation of unstable α-Gal A in plasma pH. This mutated enzyme preserves ~60% of its physiological activity in lysosome, whose pH is 4.6. This is called enzymatic pseudodeficiency. It has been demonstrated that carriers of D313Y mutation have a diminished α-Gal A activity in plasma while its intracellular activity remains preserved.[10] Thus, if the dosage of α-Gal A enzymatic activity in leukocytes, and not only in dried blood spot, could have been performed, it would have been normal.

Most clinical studies have supported the hypothesis that D313Y mutation is not pathogenic.[11] Lyso-globotriaosylceramide, a substrate of globotriaosylceramide molecule, exerts a well-established role in Fabry disease pathogenicity, therefore considered a biomarker of disease severity and prognosis.[12] Its plasmatic levels are increased in patients with mutations that are proven to be pathogenic, but not in D313Y carriers.[12] Otherwise, it has been suggested an association between D313Y mutation and white matter lesions.[10]

The physical examination did not reveal any clinical sign of the disease, such as angiokeratoma and cornea verticillata. Importantly, the patient was using chloroquine, which can lead to the development of cornea verticillata, also described as a characteristic finding in Fabry disease and present in almost all heterozygous male[1]. Other drugs that may cause cornea verticillata include amiodarone and indomethacin.[13] There is no difference between drug-related and Fabry disease-related cornea verticillata. The iatrogenic form disappears after discontinuation of the drug, though.[13] Interestingly, patients in use of chloroquine and amiodarone may develop lipidic inclusions in renal tissue similar to the those found in Fabry disease patients.[14]

The detection of low α-Gal A activity associated with high plasma or urinary levels of globotriaosylceramide or lyso-globotriaosylceramide and the identification of an affected family member with classical manifestations are criteria that establish the diagnosis of Fabry disease when doubt is present.[15] Plasma levels of globotriaosylceramide or lyso-globotriaosylceramide could not be evaluated in our patient.

His daughters, the only family members with whom he had contact, are obligatory carriers of the mutation. They had no clinical manifestation. The demonstration of tissue globotriaosylceramide deposition is considered to be the gold standard for a definitive diagnosis of Fabry Disease.[15] Our patient had been submitted to a kidney biopsy few months before starting renal replacement therapy. The microscopic findings by light microscopy were suggestive of hypertensive nephrosclerosis, without histological findings of Fabry nephropathy (Table 2).[14]

A few considerations regarding kidney biopsy in Fabry disease should be mentioned. First, histological findings may be overlooked if a biopsy is not examined

Table 2 Histological findings in Fabry nephropathy

Light microscopy
The glycosphingolipid inclusions are removed during tissue processing for paraffin embedding. Therefore, the cells, especially podocytes, parietal epitelial cells, and distal tubular epithelial cells, appear vacuolated. Hyaline-like material accumulates in the media of arteries and arterioles (Fabry arteriopathy) and sometimes in the mesangial regions.
Imunofluroescence
Negative.
Electron microscopy
Highly electron-dense multilamellar inclusions of glycosphingolipid (myelin figures made of concentric layers and zebra bodies, which have elongated striped appearance) are present in various cell types. The inclusions also stain darkly by toluidine blue on semi-thin sections. Abundant inclusions are present in podocytes, parietal epithelial cells, and distal tubular cells. Arteries and arterioles show inclusions in smooth muscle cells and hyaline-like material, consistent with Fabry arteriopathy.

by an experienced nephropatologist. Second, toluidine-blue stained is helpful to demonstrate cytoplasmic deposits. Finally, a tissue fragment for electron microscopy should always be obtained, even when this technique is not immediately available, it should be required for further investigation.

A complete investigation of Fabry disease could not be performed in the present case. Semi-thin section stained with toluidine-blue and electron microscopy, Cardiac and cerebral MRI, useful tools to detect myocardial fibrosis and white matter lesions, respectively, could not be performed. Nevertheless, based on literature review and on clinical and laboratory investigation, it was concluded that the patient does not have Fabry disease. Hence, ERT was not indicated.

Conclusion

In our patient, the D313Y mutation did not cause Fabry disease. The lack of clinical manifestations and particularly the absence of histological findings of Fabry nephropathy allowed us to exclude the diagnosis and, consequently, to not indicate ERT. The diagnosis of Fabry Disease should not be based only on the presence of low enzymatic activity. In this regard, in a recent screening among male hemodialysis patients in Bahia that enrolled 2583 patients, pathogenic GLA mutation was detected in 3 out of 72 patients with low enzyme activity, suggesting that diagnosis of Fabry Disease in males may be associated with a large number of false-positive cases (i.e., low specificity) if based only on enzymatic activity. Other important information that genotyping may provide is the type of the GLA mutation, since some misense mutations have been demonstrated to be amenable for treatment with pharmacologic chaperone.[16]

Medical practice has embraced new helpful technologies during the last decades, such as genotyping. Nevertheless, the diagnosis should always be based on a detailed medical and family history as well as on physical examination. One should be aware that, beyond indicating an unnecessary treatment, an incorrect diagnosis of a hereditary disorder may impose to patients and their families an additional emotional burden, which will certainly have a negative impact in their sense of well-being and quality of life.

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