An unexpected cause of chronic renal dysfunction and haemato-proteinuria

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**CLINICAL PRESENTATION**

A 62-year-old woman was referred to nephrology section for renal dysfunction. Initial laboratory workup was significant for a creatinine of 3.1 mg/dl, urea 119 mg/dl, and urinalysis showing haematuria (3+ red blood cells) and proteinuria (protein excretion 2+; 3.5 g/day). A renal ultrasound showed normal-sized kidneys, with sinusal hyperechogenicity.

Her past medical history was significant for smoking from a young age, arterial hypertension, dyslipidaemia and hypothyroidism. Her habitual medication was 8 mg perindopril, 5 mg atorvastatin and 0.05 mg levothyroxine sodium. There was no family history of renal disease. Serum complement levels (C3 and C4) were within the normal range; antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibody (C-ANCA), proteinase 3 anti-neutrophil cytoplasmic antibody (PR3-ANCA) and anti-glomerular basement membrane (GBM) antibodies were negative. Serology for hepatitis B, hepatitis C and HIV was negative.

By the time of the next visit she presented with a rapid decline in renal function, with an increase in serum creatinine and in serum urea to 4.3 mg/dl and 152 mg/dl, respectively.

A kidney biopsy was performed.

**HISTOLOGY**

*Figure 1, 2 and 3* represent the routine staining prepared in formaldehyde-fixed paraffin-embedded tissue in all kidney biopsy specimens: Periodic acid-Schiff, Masson’s trichrome and methenamine silver, respectively. In all of them we can observe that the glomerular tuft contains remarkably enlarged podocytes. The cytoplasm cells are finely vacuolated, lacy, with a honeycomb appearance.

No other cells in the biopsy had cytoplasm vacuolisation. Two glomeruli were globally sclerosed. Interstitial fibrosis and tubular atrophy were extensive. An arciform artery presented a substantial intimal fibrotic thickening.

*Figure 4 and 5* correspond to fresh, unfixed, unstained frozen kidney tissue.
In figure 4 we observe birefringence of lipid inclusions under polarized light.

In Figure 5 we can observe yellow auto-fluorescence of lipid inclusions under UV light.

The routine immunofluorescence performed in frozen tissue was negative.

Glutaraldehyde-fixed tissue was not available and electron microscopy was not performed.

**BIOCHEMICAL AND GENETIC STUDY**

Following the kidney biopsy specimen analysis, total blood sample was sent to the Doutor Jacinto de Magalhães Medical Genetic Centre. Biochemical study reveal that leukocyte enzymatic activity alpha-galactosidase A was partially decreased: 21 nmol/h/ml protein (normal range: 36-80). GLA gene was resequenced and the heterozygous mutation c.337T>C (p.F113L) was found in exon 2.
ANATOMO-CLINICAL DIAGNOSIS

Fabry disease

EVOLUTION AND TREATMENT

Renal function remains stable with 3.87 mg/dl of creatinine and 141 mg/dl of urea. Further study was required to evaluate the extent of the disease in other organs. The enzyme replacement was solicited and is awaiting approval.

Screening for Fabry disease was performed in the patient’s daughter. Leukocyte enzymatic activity alpha-galactosidase A was in normal range and no mutation was found in GLA gene.

DISCUSSION

Fabry disease is a lysosomal storage disease caused by an x-linked deficiency in alpha-galactosidase A enzyme (αGal) leading to accumulation of globotriaosylceramide (GL3) in many cell types. The disease affects the function of the kidney, heart, sweat glands and nerves. The clinical manifestations vary with mutation and consequent level of αGal. Female patients may show distinct phenotypes from asymptomatic to renal failure.1,2

In the formaldehyde-fixed paraffin-embedded sections the cellular lipids deposits are removed during processing and the routine stains demonstrate only vacuolated cells (Figures 1-3).

Podocytes present pale, enlarged, lacy cytoplasm with honeycomb appearance. Similar changes are present to a lesser degree in endothelial and mesangial cells and occasionally in the parietal epithelial cells. A similar vacuolated appearance could be present in tubular epithelial cells, particularly in distal tubules. Small arteries and arterioles show vacuolation of the endothelial cells and finely vacuolated areas in the smooth muscle3.

Progression of the disease leads to chronic unspecific glomerular, vessel and tubulo-interstitial lesions: mesangial increased cellularity and matrix, segmental glomerular sclerosis, capillary wall thickening, arterial and arteriolar sclerosis and tubular atrophy/interstitial fibrosis3.

Routine immunofluorescence (IgG, IgA, IgM, C3, C4, Clq, fibrinogen, albumin, kappa and lambda light chain) is negative. It could be positive but unspecific, with focal C3 and IgM deposition in sclerosed glomerular area4.

Fabry deposits are demonstrated using glutaraldehyde-fixed and epon-embedded kidney tissue3-4. With that processing the lipid deposits are not removed and can be demonstrated by toluidine blue staining in 1 micron-thick sections and by electron microscopy (EM).

Electron microscopy shows enlarged secondary lysosomes filled with granular to lamellated membrane structures that have an onion-skin-like appearance or parallel dense layers (zebra bodies). These inclusions in EM are considered the hallmark of glycolipid storage5.

Our patient has only formaldehyde and frozen tissue, without glutaraldehyde-fixed tissue, and so toluidine blue and EM could not be usefully performed to confirm the diagnosis of Fabry disease.

The storage material not removed in frozen unstained kidney tissue3,4,6 is birefringent under polarized light (Figure 4) and exhibits auto-fluorescence (Figure 5).

Affected males with classic and variant phenotypes are reliably diagnosed by the demonstration of deficient enzyme activity in leukocytes. Genetic testing is required in females and in males with borderline levels of alpha-galactosidase A activity.

Since more than 700 distinct mutations have thus far been described, identification of a mutation in a new family requires complete resequencing of the gene in an experienced laboratory7.

The genetic study of our patient was performed in a selected laboratory and one of the first mutations described as causal of Fabry disease was found8.

The lesions observed in formaldehyde-fixed paraffin-embedded and in frozen renal tissue, the partial decrease in leukocytes’ alpha-galactosidase A enzymatic activity and the heterozygous mutation c.337T>C (p.F113L) allows the diagnosis of Fabry disease in our female patient.

Disclosure of potential conflicts of interest: None declared
References


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