

Case Of the Week 7

CLINICAL HISTORY

A 42 year old male, renal allograft recipient three years back (donor brother aged 31 years), presented with progressive decline in renal functions over 6 months (rise of creatinine from 1.2 mg% to 1.8 mg%) and intermittent pedal edema.

Immunosuppression: Steroids, MMF and Tacrolimus- no history of fever, diarrhoea or drug non-compliance.

EXAMINATION:

Mild pedal edema +, BP 140/80 mmHg, Systemic examination- NAD, no graft tenderness.

INVESTIGATIONS:

Urine - Albumin 2+, no hematuria, 24 hours urine protein 1.9 gms, Urine ACR 1.8

Serum HIV, HBsAg, anti HCV, BK Virus and CMV- negative

Haematological profile -Haemoglobin 9.4 g% (94 g/L)

Urea: 49 mg% (17.49 mmol/L), Creatinine 1.8 mg% (159.1 µmol/L)

Serum Tacrolimus levels- within normal limits, Donor Specific Antibody assay- negative

USG abdomen: Normal sized renal allograft with mild increase in cortical echogenicity. Cortico-medullary differentiation maintained. A renal biopsy was performed in view of progressive rise in creatinine and proteinuria

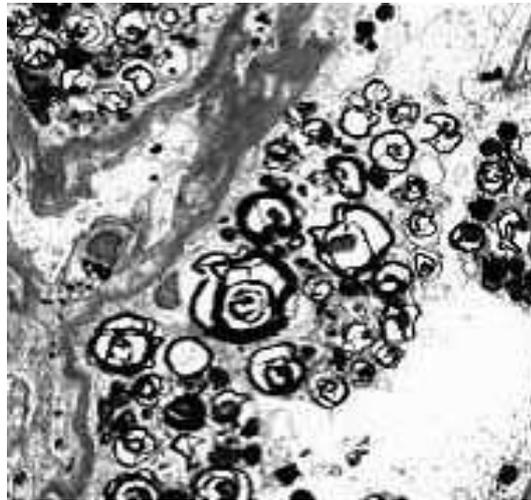
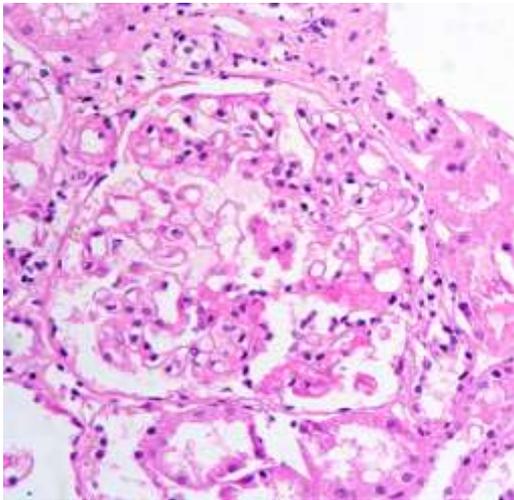
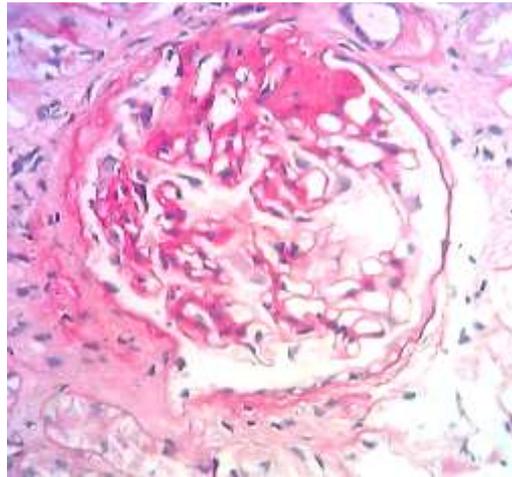
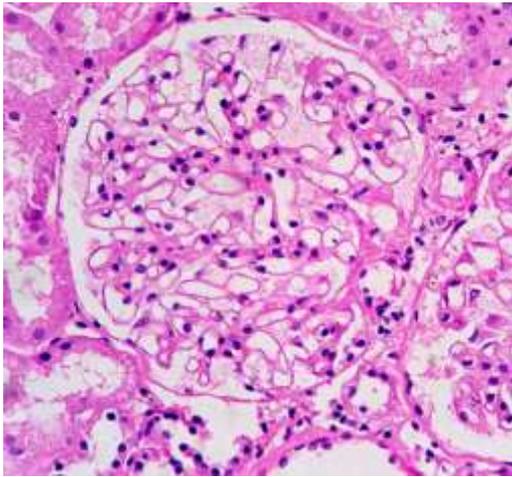
DIF studies showed non- specific segmental glomerular mesangial staining for IgM, and negativity for IgA, IgG, C3, C1q and kappa & lambda light chains.

Stain for C4d was negative in peritubular capillaries.

MICROSCOPY:

PAS stained renal allograft biopsy was available in virtual microscopy format.

Biopsy cores included both renal medulla and cortical parenchyma, containing upto 7 glomeruli. The segmental sclerosis lesions in two glomeruli are evident at low power. On higher magnification there are striking alterations in the glomerular visceral epithelial cells which are enlarged and are loaded with variably sized empty appearing vacuoles. Focal chronic interstitial inflammation and tubular atrophy/ interstitial fibrosis (Banff grade 1 are seen). There are no areas of tubulitis affecting the non-atrophic tubules and no evidence of intimal arteritis. Stain for C4d, BK virus and CMV were negative.



Ultrastructural examination of tissue retrieved from paraffin block showed osmiophilic lamellated structures in podocyte cytoplasm and focally in tubular epithelial cells.

DIAGNOSIS:

1. Banff scores: ti1, i0, g0, mm0, cg0, ci1, ct1, v0, cv1, ah2, aah0, ptc0 (Banff 2007 update). No evidence of acute or chronic “active” cellular or humoral rejection processes.
2. Fabry’s Disease involving the renal allograft, with associated (secondary) focal and segmental glomerular sclerosis in 2/7 glomeruli included in the biopsy
3. Tubular atrophy and interstitial fibrosis-Banff grade 1.

FOLLOW UP AND FURTHER INVESTIGATIONS:

1. Low serum alpha galactosidase A levels (<1 nmoles/hr/mL).

2. Further evaluation revealed that the donor (brother) who was asymptomatic with normal renal functions and no proteinuria at the time of transplantation, had in the meanwhile developed subnephrotic proteinuria and mild renal derangement (serum creatinine 1.2 mg%). The family (brother and mother) however refused renal biopsy and any further serological or genetic investigations.

The disease in all probability was donor derived and highlights two important points:

- 1. Value/ utility of donor or time zero biopsies.**
- 2. Even within the same family, Fabry disease can have clinical heterogeneity and varying rates of disease progression.**

DISCUSSION

Originally described as a dermatologic curiosity by Fabry in 1898 [1] and independently by Anderson in the same year [2], Fabry disease is an X-linked lysosomal storage disorder resulting from deficiency of the enzyme α -galactosidase A (α -Gal A), [3] leading to incomplete metabolism and progressive lysosomal accumulation of glycosphingolipids, particularly globotriaosylceramide (GL3). This causes damage to endothelial, perithelial and smooth-muscle cells of the vascular system, glomerular (visceral epithelial & mesangial cells) and tubular cells of the kidney, myocardial cells and valvular fibrocytes, epithelial cells of the cornea and ganglion cells of the dorsal root and autonomic nervous system, as well as cortical and brain-stem structures. The symptoms vary with the degree of accumulation of globotriaosylceramide in various organ systems.

The test for α -galactosidase is a fluorometric assay and uses the substrate 4-methylumbelliferyl- α -D-galactopyranoside. It can be performed in serum, isolated leukocytes and /or cultured cells. Alpha-galactosidase A deficiency is usually defined as a plasma or serum enzyme level less than 1.2 nmoles/hr/mL; normal individuals have levels above 10-12 nmoles/hr/mL, with heterozygotes having varying levels ranging anywhere from near normal to the defining limit of deficiency.

The renal pathology of Fabry disease:

Renal involvement is common in Fabry's disease and is not difficult to diagnose on renal biopsy, given the characteristic morphological alteration in most of the cases. [4]

Light microscopy: shows enlarged visceral epithelial cells (podocytes) distended with foamy appearing vacuoles, variable mesangial widening, and varying degrees of glomerular obsolescence. Within the glomerulus, the largest amount of lipid material is seen in podocytes, followed by the parietal epithelial, mesangial, and

glomerular endothelial cells. With disease progression, glomeruli exhibit mesangial widening, segmental glomerulosclerosis, and ultimately global glomerulosclerosis.

Vacuolation is also present in the capillary endothelium and distal tubular epithelial cells, including those of Henle's loop and the collecting duct, particularly intercalated cells and less commonly in proximal tubular epithelial cells. Vascular involvement includes deposition in capillary, arterial, and arteriolar endothelial cells, pericytes, and smooth muscle cells. In severe cases, there is progressive tubular atrophy, interstitial fibrosis, and varying amounts of interstitial fibrosis.

Special techniques to demonstrate the accumulated material in renal biopsies:

1. PAS, Luxol fast blue Oil red O and Sudan Black in frozen sections
2. Unstained frozen sections demonstrate birefringence, corresponding to the accumulated material in podocytes/ mesangial/ tubular cells. (Image quiz 6 www.nephro-pathology.com)
3. Biopsy fixed in glutaraldehyde embedded in Epon, and stained with toluidine blue or methylene blue shows dark blue cytoplasmic inclusions.

Electron Microscopy

Electron microscopy shows enlarged secondary lysosomes packed with lamellated membrane structures (myeloid or Zebra bodies). These inclusions can vary in appearance, from granular to lamellated, the latter being more diagnostic. The periodicity of the lamellated membrane structures when measured in routine plastic thin sections is estimated to be 4 to 5 nm, but the periodicity of their structures is 14 to 15 nm when studied by freeze fracture electron microscopy, due to better tissue preservation.

Urine Cytology

Urine cytology offers a non-invasive method to detect renal involvement in Fabry's disease. Most cells present in the urine of Fabry's patients are tubular epithelial cells (9). Levels of Gb3 can also be measured in the urinary sediment.

ESRD and Renal Transplantation in patients with Fabry's disease:

The prevalence of Fabry's disease as a cause of ESRD is probably underestimated. Few reports have described the prevalence and outcomes of Fabry disease among ESRD patients. In Europe (5) and in the United States (6), the prevalence of Fabry disease among patients on renal replacement therapy was 0.0188 (83/440,665 patients) and 0.0167 (42/250,352 patients), respectively. About 12% of ESRD patients with Fabry disease were female in both registries. In addition, several case-finding studies among ESRD populations have shown a more than 10 times

higher prevalence of Fabry disease as compared with the U.S. Renal Data System or European Renal Association-European Dialysis and Transplant Association registries [7,8]. Few of the patients in these series did not present with the typical clinical features and were therefore difficult to diagnose clinically, particularly in absence of family history or a previous renal biopsy.

Three-year survival of dialysis patients with Fabry disease was worse as compared with non-diabetic dialysis patients in Europe (60%) and in the United States (63%) [5,6]. Among kidney transplant recipients, 3-, 5-, and 10-year graft and patient survival was similar in patients with Fabry disease and matched patients without Fabry disease in Europe [5] and the United States [9]. Shah et al. [10] assessed outcomes of 197 patients with Fabry disease among 2,33,280 U.S. kidney transplant recipients and found that while 5-year graft survival was similar in patients with or without Fabry disease, a higher risk of death was seen in patients with Fabry disease as compared with a matched control population .

Recurrence of Fabry disease in renal allografts is a rare but recognized phenomenon, occurring as late as 14 years after transplantation[11-14].

Enzyme replacement therapy (ERT) in Fabry disease:

The two ERTs using recombinant or gene-activated human α -Gal A enzyme that have been evaluated in clinical trials are Fabrazyme® (algalsidase beta; Genzyme Corp) and Replagal TM (algalsidase alpha; Shire Genetic Therapies, UK). Both were approved in 2001 by the European Agency for Evaluation of Medical Products; only Fabrazyme® was approved by the FDA for use in the US. Several studies have demonstrated the efficacy of enzyme replacement therapy in retarding disease progression and improving the quality of life in these patients. [15-20]

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