Nephropathy in Fabry disease and iatrogenic phospholipidosis mimicking Fabry disease
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Fabry disease is a rare X-linked inborn error of the glycosphingolipid metabolism caused by deficient activity of lysosomal enzyme alpha-galactosidase A. It is characterized by progressive multisystemic involvement that leads to premature death due to major organ failure, particularly the kidneys and heart. It appears that the disease is underdiagnosed in patients with end-stage renal disease. Clinical presentation can be atypical and, particularly in women, subtle. Its signs and symptoms are easily neglected or attributed to other disorders and biopsy is not indicated. It can be overlooked even when biopsy is performed in early stage in women or very advanced sclerotic stage, especially by an inexperienced pathologist looking at conventional light microscopic slides of inadequate quality and when electron microscopy is not done on a regular basis.

Enzyme replacement therapy, which appears to reduce the harmful storage of undegradated sphyngolipids in cells, as well as potentially preventing the development and/or stopping the progression of secondary chronic sclerosing changes, is a revolutionary innovation. Early clinical diagnosis, standardized histopathology scoring of baseline disease before therapy, as well as longitudinal assessment of responses to therapy, highlight the important role of biopsy in disease management.

The electron microscopy appearance of intracellular undegradated sphyngolipid deposits in the form of myelin-like cytoplasmic inclusions, with a peculiar distribution pattern within nephron structures, has been assumed to be typical and diagnostic for Fabry nephropathy. However, strikingly similar cytoplasmic inclusion bodies and the same distribution pattern have been presented in the kidney biopsies of 4 case reports in the literature since 2003 ascribed to iatrogenic injury as a side effect of anti-rheumatic therapy with chloroquine. Few physicians are aware of the potentially harmful effect of this drug, widely introduced as a safe treatment of mild forms of some autoimmune connective tissue diseases. A pathologist assessing the kidney biopsy of such a patient independently of clinical information about therapy can make a wrong diagnosis of Fabry disease or overlook diagnostically relevant cytoplasmic inclusions when storage is limited and electron microscopy is not applied.

Fabry disease

Gender and a type of alpha-galactose A gene mutation play a major role in clinical manifestations and course of Fabry disease. However, it has become evident that clinical manifestations and course may differ among members of the same family, probably influenced by fairly enigmatic non-genetic factors. Over 300 mostly point (missense and nonsense) and mostly private mutations have so far been demonstrated by molecular genetic studies. A classic phenotype in hemizygous males is fairly typical, already presenting in childhood or adolescence with intermittent acroparesthesias, gastrointestinal symptoms, corneal and lenticular opacities and angiokeratoma. Later, with increasing age, gradually increasing proteinuria with renal impairment almost invariably progresses to end-stage renal disease by adulthood in the fourth or fifth decades. Death can also occur from severe cardiac involvement or cerebrovascular disease. Atypical male variations have a milder phenotype. In the past, most “carrier” females were usually thought to be clinically unaffected. Although generally presenting with a milder or even asymptomatic clinical form, full-blown disease as severe as in affected males can occur. Random inactivation of one chromosome X in the cells of various tissues and organs (Lyonization) explain the phenotypic heterogeneity in females. Assessment of the alpha-galactosidase A level in the plasma and leukocytes is today a widely
applied laboratory test for diagnostic confirmation and screening of Fabry disease. In hemizygous males with the classic phenotype, there is typically an absence or very low level of residual enzyme activity. However, female heterozygotes express highly variable, up to normal levels of the enzyme. Mutational gene analysis is therefore necessary to confirm the female carrier state.

The histopathology of Fabry nephropathy in adult hemizygous male patients has been extensively studied and characterized as diagnostically significant, especially if complementary techniques are applied. However, systematic larger cohort studies relating to the nephropathology in heterozygous females, and especially children, are still limited. Our experience is based on extensive clinico-pathological studies of 11 adult patients, of an age range 25-55 years, members of 7 families with defined alpha-galactosidase A gene mutations. Characteristic cytoplasmic deposits of GL-3 are removed during processing and in routine paraffin sections heavily overloaded cells appear enlarged and vacuolated (Fig. 1A). In adult heterozygote male patients, there is a diffuse affection of all kidney cells, with a characteristic distribution pattern. The most heavily affected are usually permanently vacuolated podocytes and distal tubular epithelial cells, followed in our experience by vascular smooth muscle cells and parietal epithelial of the Bowman’s capsule. Scattered dense cytoplasmic inclusions in the proximal tubular epithelial cells are usually barely visible. In frozen sections, cytoplasmic GL-3 deposits are preserved and show autofluorescence and birefringency under a polarized microscope. The material is also preserved in semi-thin sections as characteristically intensively toluidine blue or Azur II stained larger lamellated and smaller dense granules (Fig. 1B).

The electron microscopic appearance is even more characteristic. Heavily overloaded podocytes and distal tubular epithelial cells usually contain the largest cytoplasmic coarse lamellated osmiophilic myeloid or zebra bodies (Fig. 1C), showing a crystalline substructure with a 3-5 nm periodicity on a very high magnification. Such periodicity can also in our experience be regularly demonstrated in the significantly smaller dense granules. Intracytoplasmic inclusions characterizing Fabry nephropathy are usually regarded as surrounded by a unite membrane, but the latter is not always clearly visible. The affection of the same type of renal cells appears fairly uniform in heterozygous males, except for occasional irregular affection of the tubular epithelial cells of the distal nephron. Although only through case reports or small cohort studies, mostly published recently, it has become clearly evidenced that the distribution pattern of specific cell involvement in Fabry nephropathy of heterozygous women is usually milder than in hemizygous males, but characteristically irregular or multifocal, which is in line with the mechanism termed Lyonization.

Despite the still very limited number of case reports and only one recent small cohort study, there is convincing evidence that GL-3 intracellular storage starts early, already in the first years of life, intensifying gradually with increasing age. It has also been well established that, in addition to specific intracellular GL-3 deposits, there are histopathologic changes in Fabry nephropathy that are very much in line with clinical progression towards end stage renal disease. It can best be designated as a progressive form of secondary focal segmental glomerulosclerosis. There is almost no doubt about a causal relationship with pathological GL-3 intracellular storage, although the pathogenesis remains speculative. Focal segmental and global glomerulosclerosis, interstitial fibrosis, accompanied by tubular atrophy, arterio-, and arteriolohyalinosis, represent the standard combination of sclerosing lesions involving all nephron compartments. There is evidence that the sclerosing process is concomitant to pathological GL-3 intracellular deposits, starts in childhood and progresses gradually to end-stage renal disease. The most intensively affected nephron compartments by GL-3 deposits appear to be prone to cell injury, apoptosis and desquamation, as well as consequent sclerosis.
Podocytes particularly heavily overloaded with GL-3 deposits in Fabry nephropathy share similarities with other podocytopathies, not only due to associated focal segmental glomerulosclerosis but also clinically, with proteinuria progressing to nephrotic syndrome. Vascular involvement shares some similarities with calcineurin inhibitor nephrotoxicity and there are not only histological but also clinical laboratory findings that are in accordance with the predominantly distal tubular affection in Fabry nephropathy. In conclusion, there are several findings that accord with a hypothesis about the cytotoxicity of undegradated glycosphingolipids in Fabry disease but the exact mechanism needs further exploration. Significant progress has been achieved by the International Study Group of Fabry Nephropathy through its recent development of a standardized scoring system for a quantitative evaluation of both disease specific lesions and general lesions of progression in Fabry nephropathy, useful for baseline staging as well as for longitudinal assessment of prognosis and response to therapy.

Iatrogenic phospholipidosis mimicking Fabry disease

Renal biopsy reliability has been questioned due to four recently published case reports demonstrating similar histomorphologic changes to those of Fabry nephropathy, including characteristic cytoplasmic lamellar inclusion bodies in four patients on long-term chloroquine therapy for autoimmune connective tissue diseases. Chloroquine was introduced in human medicine as an antimalarial drug but has also been widely used over decades as a safe anti-rheumatic medication. However, since 1948 side-effects in various organs have been reported such as chloroquine retinopathy, neuropathy and myopathy. Furthermore, since 2003 four case reports have been published that have drawn attention to iatrogenic chloroquine-induced lipidosis mimicking Fabry nephropathy. To the best of our knowledge, our study is the first presented investigation of a small series of cases.

Our kidney biopsy files from the last 20 years (1987 – 2007) included clinical data and biopsy finding of 25 patients (21 women and 4 men) who were treated with chloroquine, for first week 500mg/d and the remaining time 250mg/d, mostly with a diagnosis of systemic lupus erythematosus with a clinically low activity or lupus-like systemic autoimmune connective tissue disease. A Fabry nephropathy mimicking histopathology, including characteristic ultrastructural features, was demonstrated in 6 (24%) of them, all female patients with an age range 15-66 years. They were treated with chloroquine, with a cumulative dose of 5.3g to 413g over a period of 11 days to 5 years. The kidney biopsies of 19 out of 25 patients showed no features of lipidosis, or only focal suspicious cytoplasmic dense inclusions in three of them. However, in the great majority of them, chloroquine had been withdrawn months or years before biopsy was performed.

In all 6 cases, showing Fabry disease mimicking histopathology, cytoplasmic lysosomal inclusions comprised various quantities of usually smaller dense bodies, larger concentrically lamellated myeloid bodies and straight parallel arranged lamellated zebra-like bodies. Furthermore, the homogenously appearing content of the cytoplasmic inclusions showed on higher magnification a delicate crystalline fingerprint mimicking substructure, similar to that observed in Fabry nephropathy cases, with a periodicity between dark lines of about 5nm. Furthermore, practically all renal cells were found to be involved to different extent with a distribution pattern similar to that in Fabry nephropathy with a dominant affection of podocytes and epithelial cells of distal tubules followed by glomerular parietal epithelial cells and smooth muscle cells of the vessel walls. The quantity of cytoplasmic inclusions shared similarities with female heterozygote mild form of Fabry nephropathy and even irregularities in a distribution pattern and in cell involvement were usually similar.
Six cases of our files declared as Fabry disease mimicking iatrogenic, chloroquine-induced phospholipidosis showed mostly milder forms of lupus nephritis and occasionally focal sclerosing lesions that could also be ascribed to the side-effects of medication. Furthermore, by electron microscopy in addition to already described lamellated cytoplasmic inclusions in peculiar curvilinear bodies, characteristically expressing an intermingled twisted microtubular substructure (Fig. 1E, F) were found after a careful search in 4 of 6 our cases, in three in the vascular smooth muscle cells (fig. 1F), in two within podocytes (Fig. 1E) and cytoplasm of the distal tubular epithelial cells and in one in a suspected still preserved mesangial cell of the globally sclerosed glomerulus. They were inconstantly surrounded by unite membrane and occasionally occurred with a combined lamellated and twisted microtubular substructure (Fig. 1E, F).

A variety of more than 50 other drugs with different therapeutic actions, including the anti-arrhythmic drug amiodarone, the aminoglycoside antibiotic gentamycin, several anti-depressant and anti-cholesterol agents showed similar side-effects by inducing iatrogenic lipidosis in experimental animals and in humans, having in common that they are cationic, amphiphilic and lysosomotropic. It appears that the predominant organ involvement varies with regard to the drug applied, e.g. lung involvement with amiodarone and renal predominantly proximal tubular injury with gentamycin.

Phospholipidosis after chloroquine medication appears to be systemic as shown in experimental studies, few other case reports and confirmed by skin and particularly skeletal muscle biopsy in one of our cases. After one year of withdrawal of chloroquine, a disappearance of myeloid inclusion bodies in repeated kidney and skeletal muscle biopsy was demonstrated in one of our six cases, and a clinical improvement in two of them, which is in line with some other observations on experimental animals and limited case reports in human patients. First appearance of ultrastructural myeloid cytoplasmic inclusions was demonstrated in one of our cases after 11 days of chloroquine medication with a cumulative dose of 5.3 g only, which appears to be a unique observation in human. We have not observed vacuolated macrophages in the glomeruli as reported in a case published by Albay et al 2005, but not confirmed by three other case reports. However, curvilinear cytoplasmic inclusion bodies, first described in chloroquine-induced phospholipidosis in the kidney biopsy of one patient by Müller-Höcketz et al in 2003, but not confirmed by three other case reports, were demonstrated in various renal cell types in four of our 6 biopsy documented cases. They have never been reported in Fabry disease which has been confirmed also by our study. Curvilinear inclusion bodies have been first described and appear to be typical for some types of ceroid neuronal lipofuscinosis, another lysosomal storage disease different from Fabry disease. According to our study, curvilinear bodies appear to be a clue feature which enable on structural level to differentiate between Fabry nephropathy and chloroquine-induced nephropathy in the context of systemic iatrogenic phospholipidosis.

Pathogenesis of iatrogenic phospholipidosis and chloroquine-induced remain inconclusive. However, there are arguments supporting the proposed mechanisms common to cationic amphiphilic lysosomotropic drugs:

1. Absorption of the drug by the plasma membrane and accumulation of a lysosomotropic drug within lysosomes, which seems to be a prerequisite;
2. Formation of amphiphilic cationic drug – polar lipid complexes resistant to digestion;
3. Inhibition of lysosomal enzyme activities through drug-induced increase of pH;
4. Directly by drug induced strong but reversible inhibition of lysosomal phospholipases A and C, and possibly other lysosomal hydrolases, which may play a major role in pathogenesis of drug-induced phospholipidosis different from Fabry disease as an inborn deficiency limited to one specific hydrolase – alpha-galactosidase A.
Figure 1. A-C. Kidney biopsy from a 26-year-old male patient T.Š. showing globally enlarged and vacuolated podocytes (A: PAS), overloaded with azurophilic lamellated inclusions (B: Semithin section) and large osmiophilic myeloid bodies (C: Electron micrograph) D. Osmiophilic myeloid cytoplasmic inclusion bodies only in scattered glomerular epithelial cells in kidney biopsy from a 46-year-old heterozygote Fabry patient M.Š., mother of the patient T.Š. E-F. Kidney biopsy of a 66-year-old female patient, during the last 18 months on chloroquine medication, showing myeloid bodies and one zebra body, including curvilinear profiles in the cytoplasm of a podocyte (E) as well as curvilinear and myeloid cytoplasmic inclusions (F) in an arteriolar smooth muscle cell (E-F: Electron micrograph).

References
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