SINGLE-CRYSTAL X-RAY DIFFRACTION (SC-XRD) AND SCANNING ELECTRON MICROSCOPY - ENERGY DISPERSIVE X-RAY ANALYSIS (SEM-EDX) FOR THE IDENTIFICATION OF CALCIUM OXALATE DEPOSITION IN A RENAL BIOPSY

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INTRODUCTION AND AIMS: Oxalate nephropathy is a rare entity with poor renal prognosis[1]. When history of kidney stones and/or classical risk factors for secondary hyperoxaluria (such as bariatric surgery, excessive dietary intake, pancreatic insufficiency[2]) are present, the diagnosis is easy. Sometimes the clinical onset is confusing and the diagnosis can be delayed, even if a renal biopsy is performed. We present the case of a 69-year-old man with history of type 2 diabetes and chronic kidney disease who presented with worsening renal function (creatinine 2 to 4 mg/dl), hyperkalemia and metabolic acidosis. Urine analysis showed mild proteinuria (200mg/day) and no crystals. Kidneys had normal volume and no stones were seen by ultrasonography. A renal biopsy showed mild nodular diabetic glomerulopathy and acute tubular injury with intratubular deposition of birefringent crystals (Figure 1). Differential diagnosis included oxalate nephropathy and, less likely 2,8- dihydroxyadenine and urate crystals. Since conventional spectrometry could not be performed on single crystals due to their small size, we decided to investigate the crystals with the typical structural and compositional characterization techniques used in the field of Material Science, SC-XRD and SEM-EDX.

METHODS: Single birefringent crystals were isolated from unstained sections of frozen kidney tissue using laser capture. Crystals were then secured on glass capillaries for further analysis. SEM images were collected using a FEI Quanta TM 250 instrument equipped with an energy-dispersive spectrometer (EDS). Single crystal XRD experiments were performed with Mo Kα radiation on a Bruker AXS Smart diffractometer, equipped with an APEX II CCD area-detector with 180 s of acquisition time per frame. The International Center for Diffraction Data (ICDD) powder diffraction database was used as a reference.

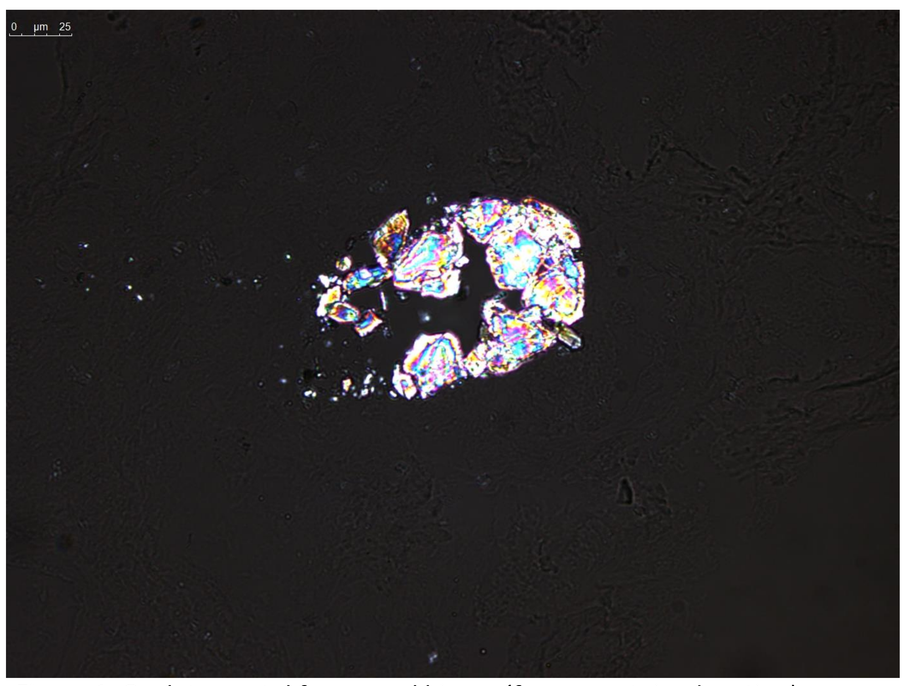

Figure 1. Oxalate crystal from renal biopsy (frozen unstained section). 40x magnification, polarized light

RESULTS: The SEM image (Figure 2) shows sharp edges, likely corresponding to the faces of a crystal embedded in the organic matrix. EDX analysis indicates carbon, oxygen and calcium as the primary elements, in agreement with the presence of $CaC_2O_4*H_2O$. A reliable quantitative analysis is prevented by the organic matrix, affecting the detection of both carbon and oxygen signal from the crystal only. Sparse diffraction spots characteristic of a single crystal were detected at 2θ values not exceeding 20° due to the small size of the crystals. The d-spacing of the reflections detected was 5.7, 3.5, 2.9, 2.3 Å, matching the strongest intensities of the whewellite phase of calcium oxalate monohydrate ($CaC_2O_4*H_2O$), ICDD 020-0231 (Figure 3).

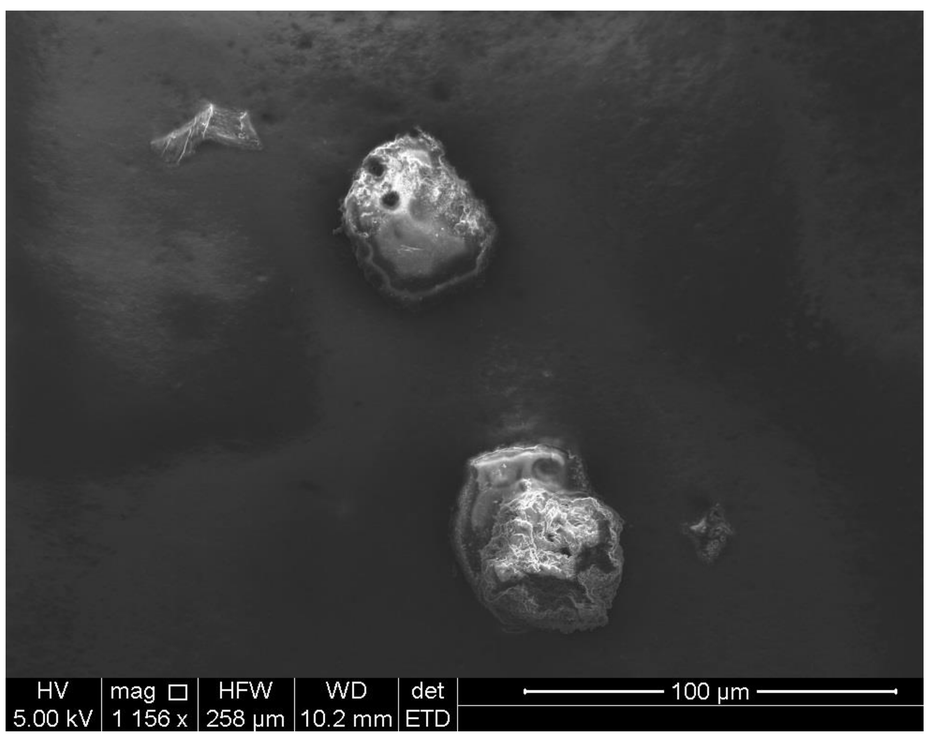

Figure 2. Oxalate crystal from renal biopsy. 1156x magnification, SEM

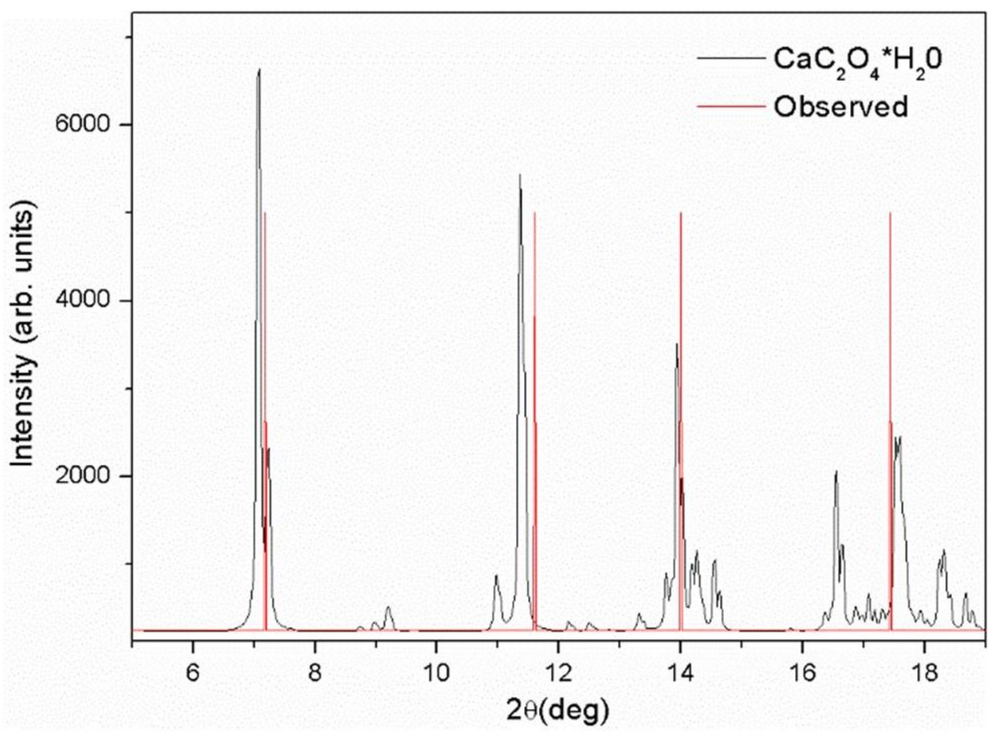

Figure 3. Spectrum of X-ray diffraction of oxalate crystals. Blue lines represent expected signals for $CaC_2O_4*H_2O$; red lines are the signals obtained from the studied crystals. Intensity is expressed in arbitrary units.

CONCLUSIONS: Oxalate nephropathy has poor kidney outcome. Characterization of the crystals can be tricky, leading to diagnostic delay. When only small single crystals from the renal tissue are available, SC-XRD and SEM-EDX studies succeeded in recognizing calcium oxalate crystals. To our knowledge, this is the first time this technology is employed for diagnostic purpose

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DOI: 10.3252/pso.eu.54ERA.2017