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Letter to Editor

Multidisciplinary investigation of an ancient renal stone in a mummy from Popoli, central Italy

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Summary

The renal stone found in the natural mummy of an anonymous nobleman dating to 19th century was investigated using advanced imaging modalities and analytic investigations. By this multidisciplinary approach we were able to identify the chemical components and their distribution throughout the sample. These results allowed to understand the lifestyle habits of the subject, as well as the exact pathogenesis of his disease.

Key words: paleopathology, nephrolithiasis, calcium oxalate, hydroxyapatite, correlative microscopy

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Dear Editor,

Urolithiasis is an ancient disease, with the earliest documented case dating to 6500 BC [1]. Bladder calculi were relatively frequent in antiquity, whereas renal colic from upper tract stones is scarcely mentioned in historical sources [2], and renal stones have been rarely described in paleopathological literature with only 20 reported cases [1,3]. The most ancient case of nephrolithiasis dates to 3300 BC [1]. The differences between bladder and renal stones may be easily explained by the different etiology, composition, and epidemiology of these two conditions[1]. Bladder stone disease is still endemic in rural or impoverished areas, while renal stone disease represents a widespread affliction of the industrialized countries in Western world [4]. In fact, bladder calculi depend on nutritional deficits of animal proteins with high percentage of vegetables, and renal stones are mainly caused by high intake of animal protein and refined sugars coupled with a low vegetable diet [1,4].

Here we present the multidisciplinary investigation of a renal stone recovered from a 19th century natural mummy found in the Church of the Holy Trinity in Popoli (Abruzzo region, central Italy) [5]. The subject was a 35-40 year old nobleman and a leading member of the Holy Trinity laymen congregation, with significant caries and periodontitis, recurrent pneumonia, and a left renal stone[6]. The endoscopic extraction of the latter yielded a brownish ovoid mass measuring 22 x 16 x 15 mm, and prompted us to apply modern investigation techniques in order to gather information about renal stone disease in past times.

The stone surface was investigated with binocular stereomicroscopy (BSM) using a LEICA S8APO stereomicroscope and scanning electron microscopy (SEM) also with energy dispersive X-ray analysis (EDX) using a Philips XL30/CP scanning electron microscope equipped with OXFORD-IncaEnergy microanalysis. Multiple tiny fragments from surface and inner portions of the calculus were submitted to X-ray diffraction (XRD) analysis with a Philips X'Pert PW 1830 X-ray diffractometer. Finally, the stone was mounted and acquired in a SkyScan 1174 micro-computed tomography (μCT) compact scanner with a voxel size of 30 mm (X-ray voltage 50 kV). The scans were over 180 degrees with a 0.4 degree rotation step. Image reconstruction was carried out employing a modified Feldkamp algorithm, using the SkyScan Nrecon software. Beam hardening correction and Fourier transform based ring artifact reduction were applied to the reconstructed 3D images.

The external surface, examined by BSM, showed a central ovoid with small superficial spherical buds (Fig. 1A), whereas the cut surface showed a core composed of sharp-edged crystals, surrounded by concentric pale and dark brown laminations (Fig. 1B). SEM observation confirmed such findings (Fig. 1C-D). Chemical elements detected by microanalysis were C, O, N, Ca, P, K, S, Cl, and Na with different distribution throughout the inner and outer surface of the stone (Fig. 1E). XRD analysis revealed 90% calcium oxalate monohydrate (whewellite) and 10% calcium phosphate (hydroxyapatite) (Fig. 1F). No trace of uric acid was found in the material. The internal structure detail investigated by μCT revealed aggregates of different density values. 3D reconstruction with color graphics allowed to render whewellite, hydroxyapatite, and external organic component in different colours. Whewellite and hydroxyapatite were equally distributed throughout the core, with laminations and buds almost exclusively composed by whewellite. (Fig. 1G).

Urinary stones are classified according to their chemical composition. Calcium stones represent about 80% of all urinary calculi, and account for calcium oxalate (CaOx) (50%), calcium phosphate (CaP, also known as apatite) (5%), and a mixture of both (45%) [4,7]. CaOx is found in the majority of kidney stones and exists in the form monohydrate (COM, mineral name: whewel-

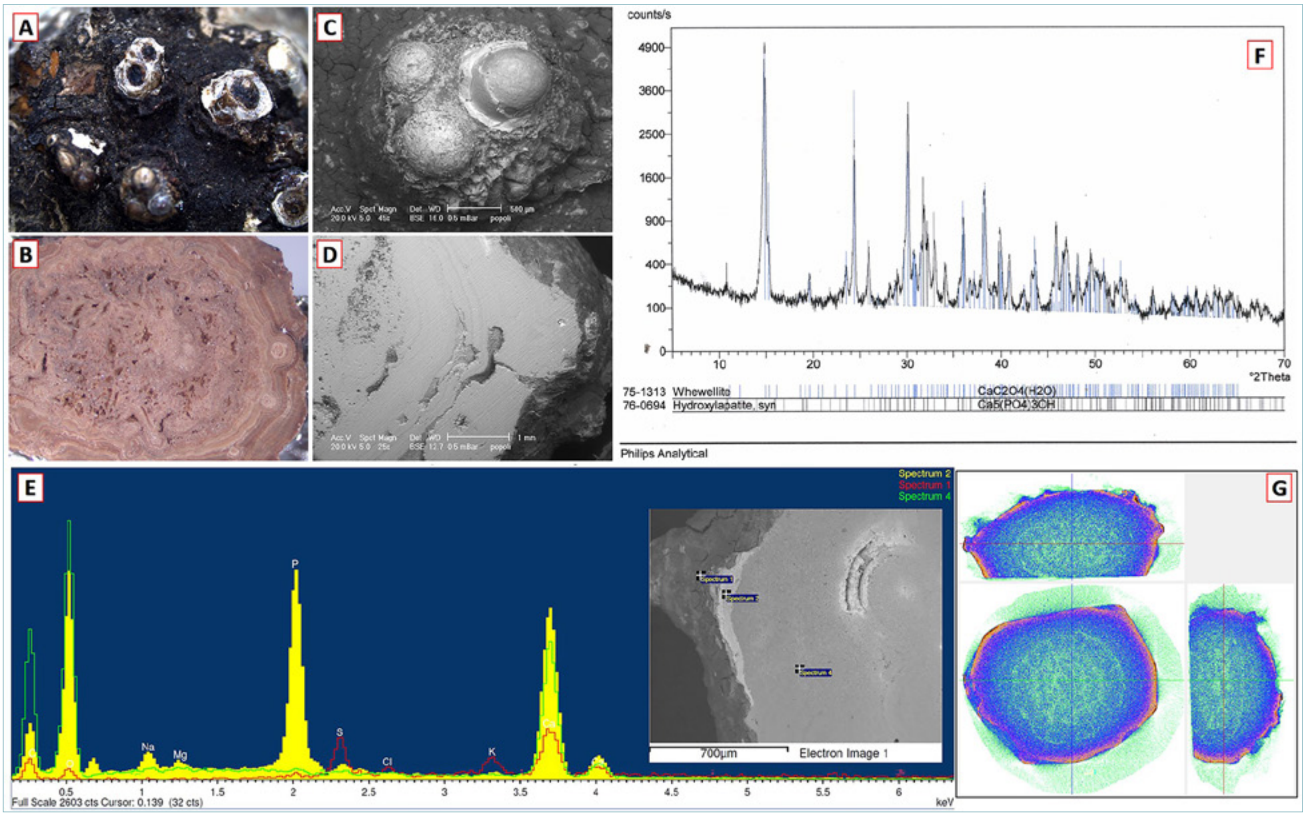

Figure 1. Binocular stereomicroscopy of the (A) external and (B) cut surface; scanning electron microscopy of the (C) external and (D) cut surface; (E) microanalysis results with the first three peaks on the left indicating C, O, and N; (F) X-ray diffraction analysis results; (G) mCT scanning with 3D reconstruction, showing whewellite in blue, hydroxyapatite in green, and external organic component in orange/violet.

lite), and dihydrate (COD, mineral name: weddellite), or as a combination of both. COM is more frequently observed than COD in clinical stones [4]. CaP is the other main constituent of calcium stones, and may be present in the form of hydroxyapatite, carbonate apatite, and brushite [4].

The formation of kidney stones (calculogenesis) is a complex and multifactorial process including intrinsic (age, sex, heredity, anatomical abnormalities) and extrinsic (geography, climate, dietary, mineral composition, and water intake) factors[4]. Factors contributing to CaOx stone formation are hypercalciuria (resorptive, renal leak, absorptive, and metabolic diseases), hyperuricosuria (uric acid interferes the solubility of CaOx), hyperoxaluria, hypocitraturia, hypomagnesuria, and hypercystinuria. CaP production is usually caused by urine alkalinization as a result of urinary tract infection with urease-producing bacteria (e. g. Proteus mirabilis). Urinary pH of 5.0 to 6.5 promotes CaOx stones, whereas CaP stones occur when pH is greater than 7.5 [4].

Over the past decades, laboratory assessment of urinary stones has been typically conducted using destructive biochemical analysis. These methods usually identified only the primary chemical content, underestimating the complexity of stones. The multidisciplinary approach employed in our case was based on advanced imaging modalities and analytic investigations [7-9], enabling us to establish the exact composition of the renal calculus. The presence of COM supports the hypothesis of excessive animal protein and salt intake, as well as deficiencies of chelating agents like citrate, fiber, and alkali foods[4]. This confirmed that the subject belonged to a high social class, as indicated by his clothes, burial location, and the presence of dental caries, whereas the absence of major arthritic changes suggested he was not subject to extensive labor [6]. The finding of CaP may be explained by recurrent urinary tract infections [4]. The almost equal mixture of CaOx and CaP in the core proves the role of the latter in the initiation of the stone formation and its first grow. The well-organised outer layers and buddings almost entirely composed of CaOx gave the calculus the typical conformation of a COM-apatite mixed stone (mulberry stone) [8,10]. Therefore, we argue that this well-nourished man had recurrent urinary infections in the first stage of calculus development, followed by subsequent stratifications due to dietary factors alone. To the best of our knowledge, this is the first ancient urinary stone submitted to μCT scanning. This represents a 3D X-ray imaging on a small scale with massively increased resolution, and proved to be extremely useful in the classification of renal stones by their microstructure and distribution of mineral components [7,10].

In conclusion, the extensive investigation by advanced imaging and analytic techniques of ancient renal stones may be considered useful to understand lifestyle habits and diseases in the past. Moreover, it must be remembered that stones recovered from natural mummies are not affected by soil/ambient contamination, yielding more reliable results than museum or osteoarchaeological materials.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

LV conceptualized the investigation, collected data, and wrote the manuscript. LA performed BSM and SEM-EDX. RQ performed XRD. MC performed micro-CT. All Authors approved the version to be published.

Ethical consideration

No ethical considerations needed.

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