A 2000-YEAR-OLD BLADDER STONE FROM GYŐR, HUNGARY: RESULTS OF CHEMICAL COMPOSITIONAL AND NON-DESTRUCTIVE STRUCTURAL ANALYSIS

EGY GYŐRI ÁSATÁSON ELŐKERÜLT 2000 ÉVES HÓLYAGKŐ KÉMIAI ÉS RONCSOLÁSMENTES SZERKEZETI VIZSGÁLATÁNAK EREDMÉNYEI •

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Abstract

A biological object was recovered from the pelvic region of an adult man (age 60) unearthed from Győr, Újlak utca Hungary. The skeleton was dated to the Roman period based on the burial customs and stratigraphic position. The find was identified as a bladder stone on morphological, radiographic, and chemo-analytical grounds with the help of traditional lab tests, X-ray diffraction, confocal Raman spectroscopy, and microscopic techniques. The mineralogical composition of this urinary stone was found to be calcium phosphate (apatite) and calcium magnesium phosphate (whitlockite). Bladder stone disease is endemic in poor agricultural regions where the typical diet is mostly based on grain carbohydrate consumption with scarce intake of animal protein. However, in our case the absence of skeletal alterations related to neurogenic origin, and the results of investigations carried out on a multidisciplinary approach, esspecially the presence of whitlockite clearly indicates infection by non-urease producing bacteria.

Kivonat

A Győrben feltárt felnőtt férfi (kora 60 év) medencetájékáról egy biológiai eredetű tárgy került elő. A csontvázat a temetkezési szokások és a rétegtani helyzet alapján a római korra datálták. A leletet morfológiai, radiológiai és kemoanalitikai alapon hólyagkőnek azonosítottuk hagyományos laboratóriumi vizsgálat, röntgendiffrakció, konfokális Raman-spektroszkópia és mikroszkópos technikák segítségével. A hólyagkő ásványtani összetétele kalcium-foszfát (apatit) és kalcium-magnézium-foszfát (whitlockit). A hólyagkőbetegség előfordul a mezőgazdaságilag hátrányos helyzetű régiókban, ahol a tipikus étrend főként gabonaszénhidrát-fogyasztáson alapul, állati fehérje bevitelének csekély volta mellett. Esetünkben azonban a neurológiai eredetű csonttani elváltozások hiánya, valamint az elvégzett multidiszciplináris vizsgálatok eredményei alapján a whitlockit jelenléte egyértelműen nem ureáztermelő baktériumok általi fertőzésre utal.

KEYWORDS: BLADDER STONE, APATITE, WHITLOCKITE, INFECTION

KULCSSZAVAK: HÓLYAGKŐ, APATIT, WHITLOCKIT, FERTŐZÉS

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Introduction

Bladder stones are hardened mineral clumps that form in the bladder when minerals are concentrated in the urine due to a longer residence time in the bladder. From a clinical point of view, bladder stones, regardless of their association with urinary tract infection and secondary renal complications, can cause suprapubic pain, blood emission with urine, pain when urinating, increased frequency in urination, difficulty or interruption of urination, and intermittent urination. Vesical calculi develop under certain medical conditions or due to an enlargement of the prostate in men (**Fig. 1**) usually older than 50 years (Gartner and Bruno, 1990; Steinbock, 1989; Steinbock et al., 1993). Bladder stones are crystalline or amorphous, with a variable number of organic compounds between the crystals. When sectioned most stones are visibly laminated, converging towards a central core that is usually considered to be the growth center. The laminations represent periodic variations in composition. Uric acid and ammonium acid urate make up the nuclei in most bladder stones. Other components include calcium oxalate, phosphate, and struvite (Gładykowska-Rzeczycka & Nowakowski 2014; Gładykowska-Rzeczycka et al. 2003; D'Alessio et al. 2005).

The determination of the chemical composition of bladder calculi through modern analytical methods such as Raman spectroscopy, X-ray diffractometry, and micro-CT could be important in paleo-pathological studies to obtain information about dietary changes, metabolic disorders of individuals affected by human diseases and economic conditions. The combined effects of diet, metabolism, and climate can sometimes complicate the interpretation of results. Although vesical calculi are rare archaeological finds (Özdemir et al. 2015), so far two reports of Avar Age and Bronze Age Hungarian examples are known in the literature (Szalai & Jávor 1987; Boross & Nemeskéri 1963). In this paper, we report the analysis results implemented on a ca. 2000-year-old bladder stone from Grave S-380 Győr, NW Hungary. Our research aimed first to determine if the mass was of human origin and to identify the type of biological stone based on its physical and chemical structure. Finally, we attempted to ascertain the probable causes of its formation.

Fig. 1.: Photograph of grave Str 380 from Győr Újlak utca (a), note the oval stone (bladder stone) in the pelvic region of the skeleton (b). X-ray radiograph of a 36-year-old male with vesicular calculus of ca. 10 cm diameter as an analogue to our case (c) (source: https://www.ctisus.com/teachingfiles/x-rays/387660).

1. ábra: Az Str 380-as objektum fényképe Győr Újlak utca lelőhelyről (a), ovális képződmény (hólyagkő) a csontváz medence körüli részén (b). Egy 36 éves férfi röntgenfelvétele kb. 10 cm átmérőjű hólyagkővel analógiaként a vizsgált esethez (c) (forrás: https://www.ctisus.com/teachingfiles/x-rays/387660).

Archeological and archaeostratigraphical description of the site and Grave S-380

Between 17 March and 1 July 2016, the Rómer Flóris Museum of Art and History carried out a pre-investment archaeological excavation of the entire surface of the Újlak Street, Győr, Hrsz. 3289/4. The excavation was carried out due to the planned residential development, as the site is located in the area of two archaeological sites, the Győr, Kálvária consolidated site (Ny.a. 70861) and the Győr, Arrabona Savaria Road late cemetery (Ny.a. 70863), which was known from previous excavations and finds on the surrounding plots (Szőnyi 1973, 5-67, 1974, 5-43,1979, 5-57, 1986, 5-34, and summarized in Szőnyi 1992 and Bíró et al. 2006, 71-78). The excavation of the 4623 m^2 area revealed 626 archaeological features, including 218 graves from the southern cemetery of Arrabona, dating from the 1^{st} to 4^{th} centuries AD. In 2017, further excavations were carried out on the site, revealing 134 more burials. Of these, 74 were cremations, 2 urns, and 58 skeletal burials. This brings the total number of graves to 352.

The object of study in this article is an unusual find in the Roman archaeological record, a bladder stone preserved in its original position in a grave above the pelvic bone of the deceased. The uncommon find was recovered from skeletal grave Str 380. The attachments (shoe pins) found in the grave do not allow a more precise dating within the Roman period, but the topographical and stratigraphic position of the grave provides a clue to the date of the burial. The site is covered by a continuous humus layer of varying thickness (5–350 cm). A sand mound is in the north-eastern and north-western corner of the site, with the yellow sand subsoil sloping downwards in a southerly and easterly direction. On the eastern side of the site, a large tumulus (Str 318), 38 m in diameter, was excavated, which was unique in the urban setting and which, because of its monumentality, must have been a landmark and a space-former, since the cemetery area contained no other graves except the tumulus. These burials, including Str 380, were in any case excavated after the construction of the grave. The date of the tomb and its unusual occurrence in urban cemeteries at the end of the 1st century make it probable that the ashes of a man of high rank, presumably of Italian origin, were buried there.

Around the grave, most of the other graves, including Str 380, the site of the urinary stone, were found in a narrow band of several layers. Some of the deceased were cremated (57 graves), while others were buried in coffins or shrouds (154 graves). The tomb is located on the west side of the grave, west of the pile of stakes (Str 237) and clay brick pile (Str 83) supporting the mound, in a superposition above the square ditch (Str 224) framing the grave. This suggests that the deceased of Str 380 was buried long after the grave was built and the trench framing it was filled in. The dating of this grave is supported by the position of two other graves. The pit of grave Str 380 is cut by grave Str 401, the upper part of the filling of which yielded a Drag 54 cup, whose manufacture and import dates from 150–230 AD (Gabler 2016, 123). In addition, a child's grave, Str 93, was found in a superposition above grave Str 380, with no annex and only fragmentary finds in its filling. From the above, it can be concluded that the first grave to be constructed was Str 318 at the end of the 1st century, and that it was established much later, after the filling of its trench, Str 224. The production of the terra sigillata cup of the stratigraphically important Str 401 tomb started sometime in the mid-2^{nd} century, so the Str 401 tomb cannot be earlier than this. The object similarly dates the Str 380 tomb terminus post quem, which suggests that the deceased was buried sometime in the second half of the 2^{nd} century or the 3^{rd} century. The orientation of the burial is W-E. The grave pit is 90 cm deep, rectangular with rounded corners, vertical sides, and a straight bottom. The body is in a stretched position, lying on its back, with the skull facing upwards. Both arms are bent and rest on the abdomen. The bladder stone is located in the lower part of the pelvis, between the pubic bones, slightly to the right. The tomb is poor, with only shoe nails recovered from under the feet.

Materials and methods

The classical anthropological and paleopathological analyses were carried out based on macromorphological approach implied by methods accepted and well known internationally and among Hungarian researchers as well (Ubelaker et al. 1989; Pap et al. 2009). To examine the shape, structure, and chemical composition of the stone the following methods were employed:

1) macroscopic evaluation: the length, width, and thickness were measured by a geometric compass while the circumference was measured by using a tape. The weight was determined by electronic balance;

2) to examine the morphological structure the sample was scanned at full rotation at a pixel size of 35 microns at a resolution of 1500 x 1500 pixels with a voltage of 90 kV and a current of 230 μA using a Bruker Skyscan 2211 Nano-CT scanner. For each specimen, all reconstructions were performed in ORS Dragonfly Software package;

3) the most important chemical compounds of calcium, ammonium, magnesium, uric acid, oxalate, phosphate, and cystine were determined with reagent kit Ecoline Urinary calcium analysis (DiaSys Diagnostic Systems GmbH);

4) X-ray powder diffraction analysis (XRD) was used for determining the mineralogical composition of the object besides crystalline or amorphous structure. The sample under study was ground in an agate mortar and pestle. The instrument used was a Shimadzu XRD-6000 X-ray diffractometer using a Cu X-ray tube (I: 1.5405 Å) with 40 kV, 30 mA Cu Kα radiation. Scanning was carried out for 2θ in the range 0–75°;

5) Raman confocal spectrometry was used not only for the determination of the mineralogical structure but also to test for the XRD results. A Horiba Jobin Yvon Lab Ram confocal Raman spectrometer with an Olympus BX41 microscope and Peltier cooling CCD (1024 × 256 pixels) detector was used to obtain the Raman spectra. The 780 nm laser line of a He/Ne laser was employed for Raman excitation at a magnification of 100x.

Results

Anthropological description of the skeleton S-380

The bones of a male (?) of the age group "senile" (50–x) buried in a stretched position are very fragmentary. As a consequence of this fact, the description of the skeleton is somewhat under-estimated. The maxilla, and mandibular alveoli were generally abraded; 3 teeth (upper M1, PM1, PM2) were available for dental examination. Tooth wear was very pronounced and medially oblique. Signs of osteoarthritis on the right front metacarpus are present. There is a slight bone overload on the vertebrae (degenerative spondylosis), which is more pronounced on the lumbar elements. Healed periostitis (periosteal reaction) and enthesopathy (inflammation of the ligamentous attachment site) are observable on the right humerus (corpus humeri, tuberositas deltoidea). Further traces of enthesopathy are observed on the 2 clavicles (impressio ligamenti costoclavicularis), on the anterior surface of the right patella, and the proximal epiphysis of the tibia (eminentia intercondylaris). Presumably suggestive of a healed fracture, the right caput humeri may have been slightly displaced towards the middle part of the cavitas glenoidalis and almost in line with the tuberculum majus. The fragmentary skull is not suitable for taxonomic examination.

Calculus analysis

The calcified mass was kidney-shaped, light brown colored with a few darker spots, and presented a rough, porous surface (**Fig. 2**). The calculus investigated, measured 4.0 × 3.0 cm, was longitudinally sectioned via micro-CT slices as physical sectioning was not possible during the investigation due to later conservation measures of the artifact. Its original weight was of the order of 35 g. Radiographic inspection revealed a concentric layered structure of the object, which confirmed that it was unlikely to be of geological origin but rather represented a calcification of biological origin (**Fig. 2**). The location of the calcified mass in the pelvic area, together with its shape, its considerable size, and morphological structure, points to a possible diagnosis of a bladder stone. From the CT image section, several layers could be distinguished: the outermost presented a compact matrix of high density, which gradually transformed into a granular, porous, and disorganized structure of lower densities (**Fig. 2**). The multiple mineral composition of the stone is clearly underlined by the results of micro-CT analyses, which displayed concentrical patterning of different material composition with inward changing densities.

The Raman confocal spectroscopy confirmed that the composition of the object was in hydroxyapatite $[Ca_5(PO_4)_3(OH)]$ form (**Fig. 3**). Raman spectra of calcium phosphates were dominated by strong $\nu sHPO_4^{2-}$ and νsPO_4^{3-} bands appearing in the 900–1000 cm^{-1} spectral range and the phosphate bending and $\nu asPO_4^{2-}$ bands in the 600–400 and 1100–1000 cm^{-1} range, respectively (**Fig. 3**). In Raman analysis, the frequencies of these bands depend on the anions, adjacent cations, and the crystallization of water molecules (Carmona et al., 1997). The wide base of peaks between 600–400 cm^{-1} range is characteristic of apatite of biological origin.

Based on the results of XRD measurements the most abundant minerals in the bladder stone under study are hydroxyapatite (HAP: $Ca_{10}(PO_4)_6(OH)_2$) and whitlockite (WH: $Ca_{18}Mg_2(HPO_4)_2(PO_4)_{12}$) (**Fig. 4**). Raman spectroscopic measurements could only confirm the presence of hydroxyapatite due to measurement of a micro-scale focal point of the sample in contrast to XRD giving a bulk composition (**Fig. 4**). According to the results of the chemical kit test, the presence of Ca^{2+} (6–7%), Mg^{2+} (10%) and PO_4^{2-} (15–20%) was attested from the most important chemical components (**Fig. 5**).

Whitlockite in vivo forms under acidic pH conditions while hydroxy apatite generally precipitates in neutral pH. As the samples under study derive from the surficial layers of the stone, alteration due to low soil pH cannot be fully excluded. Pure-phase WH nanoparticles are obtained under acidic conditions with excess Mg^{2+} ions, where precipitation of HAP is impeded (Jang et al. 2015). Hydroxyapatite (HAP: $Ca_{10}(PO_4)_6(OH)_2$) transforms into dicalcium phosphate dihydrate (DCPD: $CaHPO_4*2H_2O$) and then into WH in the presence of Mg^{2+} ions as the pH decreases. WH is the most stable calcium phosphate compound below pH 4.2, whereas HAP is the most stable around neutral pH. Mg^{2+} ions, which are known to block the growth of HAP, can play a key role in WH formation.

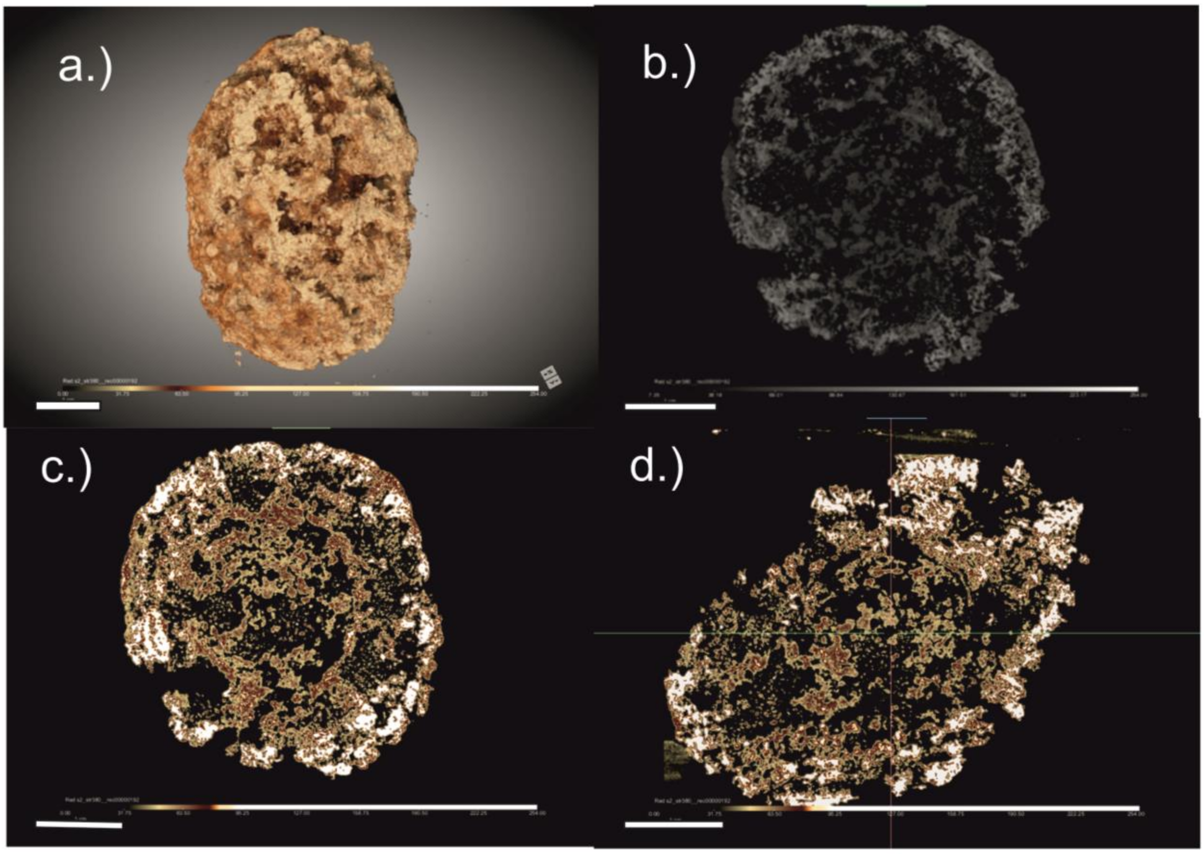

Fig. 2.: 3D reconstruction (a) and 2D CT cross-sectional (b, c) and longitudinal (d) slices of the bladder stone (note coloring. resembles closely the original, multiple composition of different relative densities and radial structural organization of the calculus under study is also visible)

2. ábra: A hólyagkő 3D rekonstrukciója (a) és 2D CT keresztmetszeti (b, c) és hosszmetszeti (d) szeletei (megjegyzés: a színezés nagyon hasonlít az eredetihez, a vizsgált kő különböző relatív sűrűségű többrétegű összetétele és radiális szerkezeti szerveződése is látható)

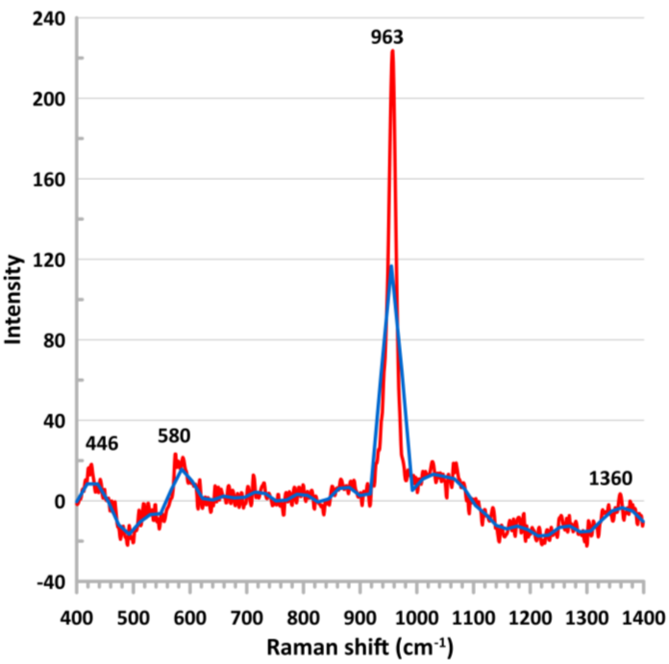

Fig. 3.: Raman spectrum of the bladder stone (note the presence of strong $\nu_s HPO_4^{2-}$ and $\nu_s PO_4^{3-}$ bands appearing in the 900–1000 cm^{-1} spectral range and the phosphate bending and $\nu_{as} PO_4^{2-}$ bands in the 600–400 and 1100–1000 cm^{-1} range)

3. ábra: A vizsgált hólyagkő Raman spektruma (jól látszanak a $\nu_s HPO_4^{2-}$ and $\nu_s PO_4^{3-}$-csúcsok a 900–1000 cm^{-1} spektrális intervallumban, valamint a $\nu_{as} PO_4^{2-}$-sávok 600–400 és 1100–1000 cm^{-1} intervallumban azonosítják a különböző foszfát csoportokat)

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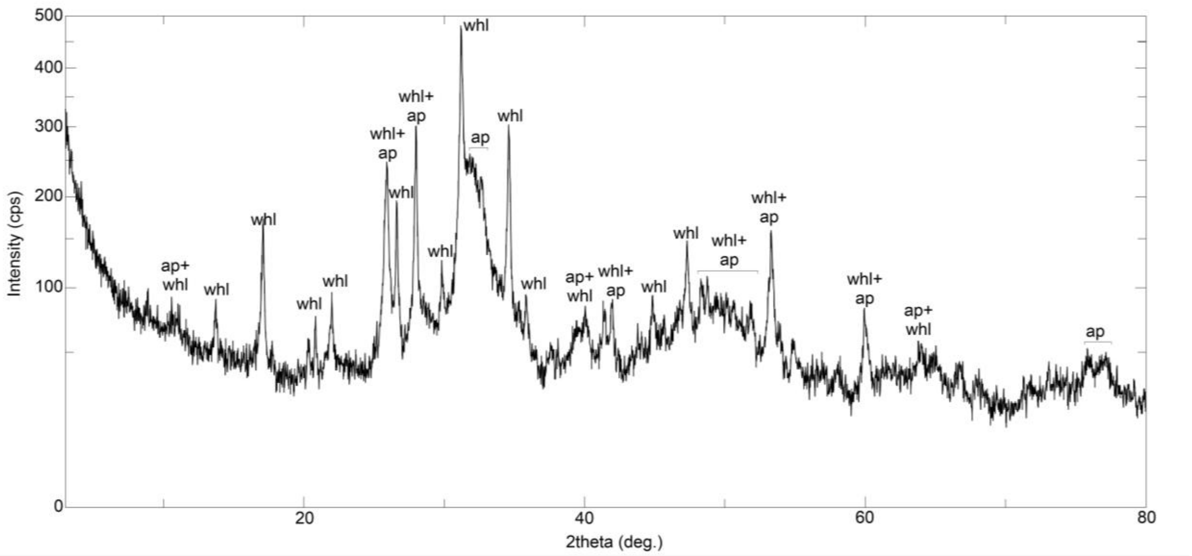

Fig. 4.: Results of XRD mineral composition analysis of the bladder stone with peaks of whitlockite and apatite

4. ábra: A hólyagkő XRD ásványtani összetétel vizsgálat eredménye whitlockit és apatit csúcsokkal

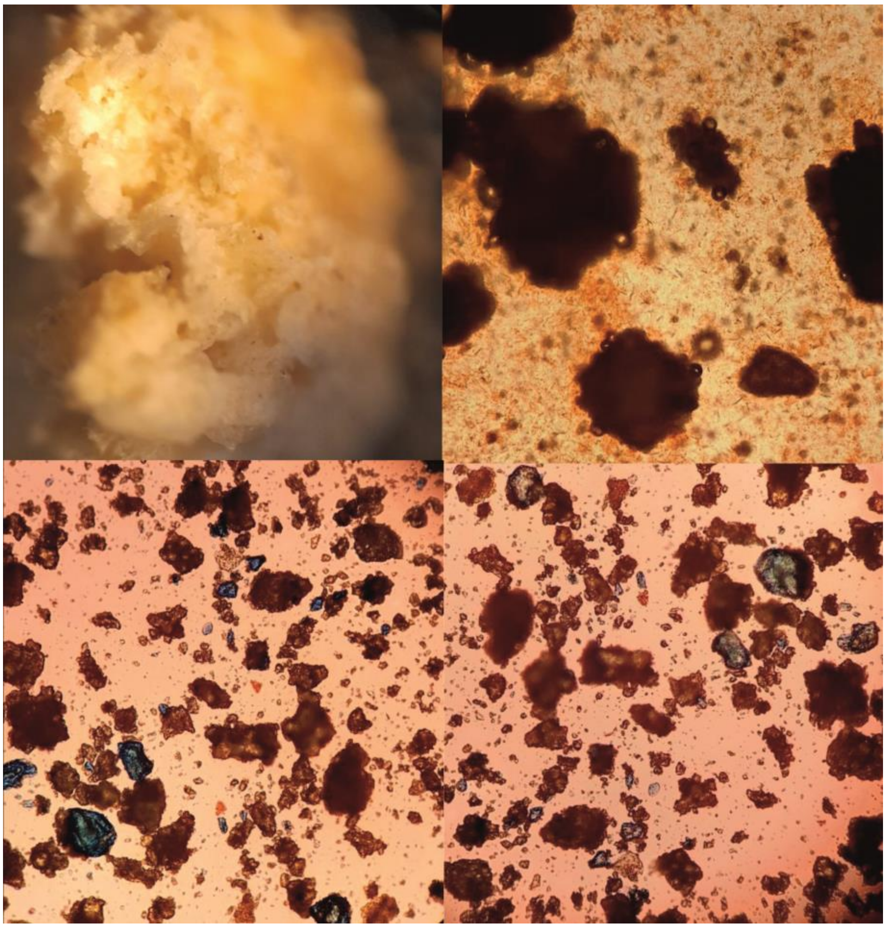

Fig. 5.: Microscopic and chemical test results of the studied bladder stone (magnifications upper left :40x and all other 100x, brown: apatite, blue: Mg-bearing hydroxyapatite)

5. ábra: A hólyagkövön elvégzett mikroszkópos és kémiai laborvizsgálatok eredményei (nagyítás bal felső kép 40x, többi 100x, barna: apatit, kék: Mg-tartalmú hidroxiapatit)

Conclusion

At present, bladder stones predominate in elderly individuals. They are associated with urinary stasis secondary to prostate hyperplasia, urethral stenosis, or neurogenic bladder dysfunction, uric acid being the most frequent component of them. Bladder stones related to diets poor in animal protein predisposed individuals to urinary tract infections.

Considering the relatively old age of the person under study, prostatic hypertrophy cannot be ruled out. Otherwise, no signs of neurological diseases/ conditions (e.g. paralysis) which can both manifest on skeletal remains and can be related to neurogenic bladder were observed during the paleopathological analysis. The previous fact, furthermore, the morphology and composition of the stone, mostly carbonate apatite with a high degree of carbonatation suggests rather an infectious, than a neurogenic origin.

Infectious urinary stones frequently originate from urease-producing bacteria (Proteus and Pseudomona are the most common) (Iliadelis et al. 1999). Urease hydrolyzes urea to form ammonium and bicarbonate, both increasing urinary pH. This urinary environment produces the formation, crystallization, and precipitation of magnesium ammonium phosphate (struvite) and a carbonate apatite (with a high degree of carbonatation). The presence of struvite and highly carbonated carbonate apatite are pathognomonic for a stone resulting from an infection (Daudon 2005, Daudon et al. 2008). The fact that struvite, characteristic of an infection-related stone, has not been detected in this case might be because struvite is unstable and decomposes with time. The acidifying effect of groundwater at the site may have played a role in the dissolution and disappearance of struvite. Despite this, the presence of the carbonate apatite with a high degree of carbonation and calcite, also observed in patients with stones due to infection, is highly suggestive of an infectious origin. Recently whitlockite has been reported from kidney stones of infectious origin as well as from non-urease producing bacteria (Bazin et al. 2022). So, the lack of struvite in our sample might be explained by infection of non-urease-producing bacteria and not secondary taphonomic disappearance. The case presented here is of interest for its antiquity and because it adds to the dataset of archaeological calcifications that extends knowledge of diseases and health in past human populations.

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Contribution of authors

Gulyás Sándor Conceptualization, Interpretation, Micro-CT analysis, Writing – Original Draft, Review & Editing. **Marcsik Antónia** Conceptualization, Anthropological description. **Gulyásné Gungl Andrea** Chemical test and microscopic analysis. **Fintor Krisztián** Raman spectroscopy. **Raucsik Béla** XRD analysis. **Nagy Réka** Material provision, Archaeological description. **Kiss Péter** Archaeological and historical data, Dating.

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