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"Aged" autopsy gallstones simulating dry bone context: A morphological, histological and SEM-EDS analysis

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ABSTRACT

Objective: The aim of this paper is to provide information on the morphology and composition of gallstones based on clinical samples in order to assist paleopathologists and bioarchaeologists in recognizing their presence in archaeological contexts.
Materials and Methods: 270 gallstones were extracted and macerated from autopsies conducted at the *Istituto di Medicina Legale* in Milan (Italy) in order to simulate a dry bone recovered from archaeological contexts. Morphological, histological, and elemental variation was documented.
Results: Gallstones vary in size, shape, color and texture. The cross-sectional surface correlates with chemical composition and is a valuable tool for classification into subcategories of stones. Histological analysis can confirm the classification. Elemental analysis yielded a higher frequency of carbon, calcium and phosphorus.
Conclusions: Although identification of gallstones in archaeological contexts can be challenging, familiarity with morphological, histological, and elemental variation can assist researchers in the field and laboratory.
Significance: Identifying gallstones in archaeological populations will assist researchers in estimating their frequency in the past and the environmental, cultural, and biological conditions leading to their presence.
Limitations: Small sample size derived from a modern and limited autopsy population may minimize the types and degree of variation present in the past. Effects of climate, soil, and taphonomy were not evaluated.
Suggestions for Further Research: Examination of larger samples derived from diverse populations may reveal greater variation or more diagnostic aspects of stones.

1. Introduction

Gallstones (cholelithiasis) are quite common, present in about 10% of individuals over 40 years and in 30% over 70 years (Siddiqui, 2016; Waldron, 2008). It is estimated that 25% of patients with cholelithiasis or urolithiasis (urinary stones) develop a recurrent stone (Johnston and Kaplan, 1993; Knoll et al., 2011; Siddiqui, 2016). Stone formation is a multifactorial condition associated with risk factors, which include female sex, advancing age (gallstones are uncommon in infants and children), high body mass index, rapid weight loss, familial history of gallstones, increasing number of pregnancies, diabetes mellitus type 2 and diet and activity (Festi et al., 2008; Shaffer, 2006). The highest prevalence of cholelithiasis is seen in Native-Americans and in decreasing order in Euro-Americans, Europeans, African-Americans and black Africans (Shaffer, 2006). These results suggest a genetic predisposition to stone formation (an estimated 30% genetic component) (Sanders and Kingsnorth, 2007; Shaffer, 2006). Cholesterol stones represent more than 75% of gallstones in western countries (Johnston and Kaplan, 1993; Siddiqui, 2016) and are caused by an "imbalance in the chemical constituents of bile" (Sanders and Kingsnorth, 2007). Indeed, when the bile becomes oversaturated with cholesterol, this excess is precipitated as solid microcrystals that accumulate and grow into stones. Pigment stones appear to be present in older individuals than cholesterol stones and are mainly composed of calcium bilirubinate, inorganic calcium salts and fatty acids (Johnston and Kaplan, 1993; Siddiqui, 2016).

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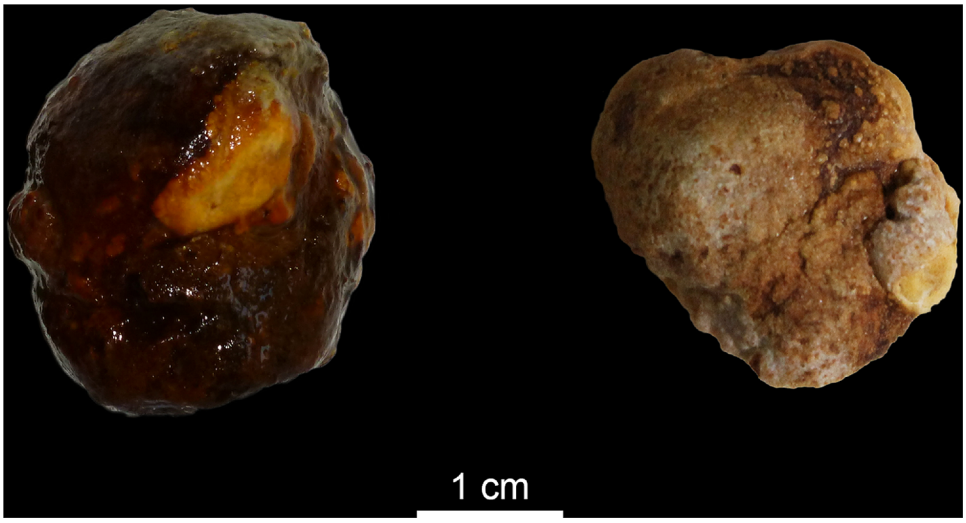

Fig. 1. Extracted gallstone from a fresh cadaver (left) and after maceration to mimic the effects of archaeological "aging" (right).

In spite of their common occurrence in modern populations, gallstones are less commonly found in the paleopathological record. Angel (1973) reported two brown faceted gallstones found between the ribs and iliac crests of a 45-55-year-old male in Mycenae, Greece, dated 1600–1500 BCE. Smith and Dawson (1924) note the presence of gallstones in a mummified priestess from Amen dated 1085–95 BCE, and Gray noted radio-opaque gallstones in a mummy of the late Dynastic period (525–343 BCE) (Gray, 1967). The mummified remains of Kha, the architect of Amhenotep III, who lived between 1460 and 1400 BCE, contained 14 gallstones (Cesarani et al., 2009). Similarly, the well-preserved body of a 50-year-old woman from the Han dynasty (*circa* 200 BCE), buried in an airtight coffin in the province of Hunan, China, revealed the presence of several gallstones (Wei, 1973). A study by Munizaga et al. (1978) showed that gallstones were recovered in 2 out of 75 mummies dated 100–300 CE from northern Chile. Detecting cholelithiasis in skeletal remains is more challenging, but gallstones have been found in a skeleton from Merovingian Germany (*circa* 750 CE), a 9th-century burial in Hérault, France, at least 6 burials in the Ohio Libben Woodland site dated 1000–1200 CE (Steinbock, 1990), and in a London grave dated 1000–1200 CE (White and Dyson, 1988). Clearly, without mummification of tissues or the recovery of stones *in situ* near the anatomical location of the gallbladder, detecting the presence of gallstones in archaeological contexts is difficult. Hence, the goal of this study was to provide researchers with an understanding of the wide morphological characteristics of gallstones in order to facilitate identification and successful recovery.

2. Materials and methods

From February 2014, to January 2017, 270 gallstones were extracted during 25 autopsies in the *Istituto di Medicina Legale* in Milan, Italy (Fig. 1). Each of the 25 autopsy extractions underwent maceration in tap water for several weeks until the calcified elements were completely separated from soft tissues and biological liquids.

The macroscopic analysis focused on several criteria, including the location where the calcified elements were found in the autopsied body, the general morphology (including shape, texture and color), and dimensions (measured with a Vernier caliper) of the stone. For the cross-sectional analysis, one stone from each autopsy extraction (a total of 25 stones) was cut transversally with either a scalpel or a small saw and processed with a horizontal grindstone (Struers DAP-7) and abrasive discs (Buehler micro cut discs, grain of 180, 320, 500, 1200, 2400 and 4000) progressively for a closer observation of the cut surface.

The histological analyses included two protocols: undecalcified and decalcified. For the undecalcified protocol, the samples were ground (using the same process as in cross-sectional surface analysis) to obtain thin sections, embedded in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and kept at ambient temperature for 72 h until the synthetic resin solidified and could be viewed with an optic microscope. For the decalcified protocol, gallstones were first fixed in formalin (v/v, PH 7–7.6, ratio 20:1 v/v) for 24 h and decalcified at room temperature in Decalc, 14% hydrochloric acid (Histo-Line Laboratories, Milan). However, the structural integrity of the stones collapsed during the xylene passage before fixing the samples in paraffin. Thus, the decalcified protocol could not be completed.

Finally, scanning electron microscopy was performed with a Cambridge Stereoscan 360 (Oxford, U.K.) with electron gun, vacuum pump, and image acquisition software, and energy dispersive spectrometry with detector from 138 eV to 5.9 keV (Oxford Link Pentafet, Oxford, UK) to provide the elemental composition of the stones.

3. Results and discussion

Each stone selected for analysis came from different extractions and thus from different individuals. Fourteen stones out of 270 calculi underwent undecalcified histological analysis and 9 underwent SEM-EDS analysis. A total of 8 gallstones underwent both histological and SEM-EDS analyses. The result of these analyses indicate that gallstones appear in a great variety of shapes, sizes and colors (Fig. 2) (Table 1). The stones observed appeared round, oval, multilobular or polygonal in shape, and presented varied external texture including smooth, porous, rough or dotted. Their coloration varied from grey, shades of white,