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Combined microstructural and mineralogical phase characterization of gallstones in a patient-based study in SW Spain - Implications for environmental contamination in their formation

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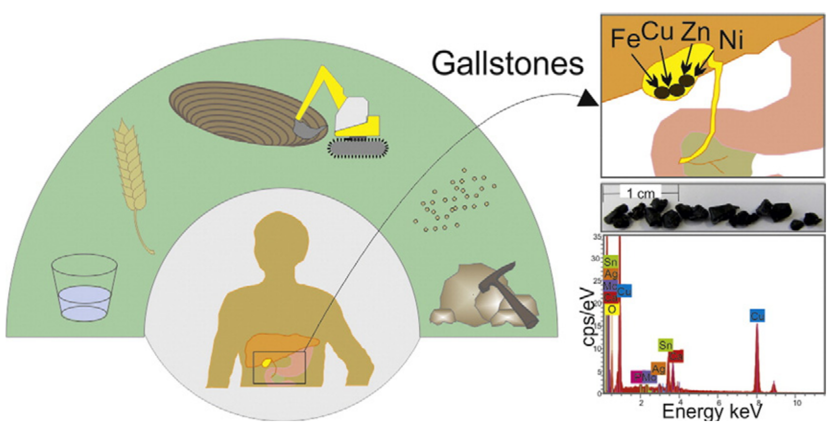
HIGHLIGHTS

- Gallstones from environmentally contrasting populations were characterized.
- Metal exposure through consumption of local water and food, atmosphere, and soils.
- Metal exposure may have an impact on the higher tendency of pigment stone formation.
- Fe, Cu, Ni, and Zn are common metal constituents in the stones from the study group.

GRAPHICAL ABSTRACT



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ABSTRACT

This study explores the environmental impact of metal exposure on humans through detailed phase and structural characterization of gallstones from two environmentally contrasting populations in Huelva Province (SW Spain). A total of 42 gallstone samples, obtained after surgical intervention at the Riotinto Hospital, were studied by powder X-ray diffraction (XRD), Fourier Transformed Infra-Red spectroscopy (FTIR), FTIR-μ-ATR (Attenuated Total Reflection) coupled with an optical microscope, and by Environmental Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy (ESEM-EDS), and subsequently classified according to their phase composition and structure. Additionally, the patients were enquired for their living habits in order to analyze the source of possible exposure to metal contamination.

The gallstones were classified into pure, mixed and composite cholesterol stones, black and brown pigment stones, and carbonate stones. The patients from the study group residing in a region with acknowledged metal contamination of both natural and anthropogenic origin have a higher risk of metal exposure through contaminated soil, particle matter in the air, and consumption of local water and food products. According to our findings, the metal exposure is related to a higher tendency of forming black pigment stones in the study group in

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comparison to the control group residing in a natural park with nearly pristine environmental conditions. Moreover, the gallstones from the study group showed to contain more abundant metal components, such as Cu, Fe, Ni, and Zn, than those from the control group. To our knowledge this is the first study to examine the regional environmental impact of metal exposure on human gallstones.

1. Introduction

Gallstones are human biomineralizations composed of organic and inorganic phases, which are formed in the biliary tract. Gallstone disease (cholelithiasis) is a common disease that affects relatively large number of people in Western World; for instance, 10–15% of the population in the United States (Stinton et al., 2010). Surgical intervention (cholecystectomy) is nowadays the best treatment method. There is no general and standardized classification system of gallstones, and the proposed classifications in the literature vary in nomenclature and categories according to the geographical location, study methods and whether composition, color and/or structure are considered (Kleiner et al., 2002; Kim et al., 2003; Qiao et al., 2013; Sharma et al., 2015; Cariati, 2015). The principal lines of classification group the gallstones into cholesterol stones, pigment stones and mixed stones. Rarer gallstones include for instance carbonate, phosphate, cystine, protein, and calcium stearate stones (Qiao et al., 2013). A proper classification system would contribute to a better understanding about the underlying pathogenesis of gallstone disease as the composition and structure of the gallstones provide knowledge about the bile conditions and processes triggering their formation.

Understanding the formation of gallstones is crucial in unraveling the intrinsic and extrinsic processes causing them, but there are still many gaps in our knowledge. Several studies have tried to relate the influence of medical conditions, obesity, diet, genetics, gender, race etc. to the gallstone formation, but the process is very complex and there are multiple risk factors (Katsika et al., 2005; Méndez-Sánchez et al., 2007; Wang et al., 2008). Recent studies reveal that metal contamination of both natural and anthropogenic origin have an adverse impact on human health (Tchounwou et al., 2012; Zhao et al., 2014; Bhowmik et al., 2015; Gu et al., 2016; Núñez et al., 2016). Environmental metal contamination probably has an influence on the gallstone formation as well, but this aspect has not been studied in detail. The growth rate of gallstones in-vivo is not well-known. There are indications that their formation takes several years with an approximate growth rate of 1 mm/year (Nudelman, 1993), making them good candidates for long-term recording at decadal temporal scale of prevailing metal exposure to humans.

This study aims at exploring the impact of metal contamination on gallstones formation by comparing two environmentally contrasting populations in the Huelva Province, SW Spain. The study population resides in an area with high metal abundance derived from the naturally metal-enriched bedrock and important historical mining activities in the region of the Iberian Pyrite Belt (central part of the Huelva Province), whereas the control population resides in the Natural Park of Sierra de Aracena and Picos de Aroche (northern part of the Huelva Province). This study bases on a detailed phase characterization and classification of gallstones from patients of both populations by using comprehensive spectroscopic and microscopic approaches.

2. Materials and methods

A total of 42 gallstone samples were obtained from patients who underwent cholecystectomy at the Riotinto Hospital in Huelva Province, which receives patients from central and northern part of the province. The patients signed a consent form authorizing the use of the calculi in this research. The samples are treated totally anonymously and the identity or any personal data of the patient are not revealed at any point. The patients were interviewed after surgery and they were asked questions related to their living habits and diet (Supplementary Data). The patients were divided into a study group and a control group according to their place of residence at the time of the surgery with the aim of tracing the impact of environmental contamination on the formation of gallstones. The prerequisite for each group was to have resided in the area at least during the previous ten years. The study group refers to patients living in the central part of the Huelva Province where there is acknowledged natural and anthropogenic metal contamination derived from massive sulfide deposits of Iberian Pyrite Belt and vast sulfide mining activities since the Third Millenium B.C. (Nocete et al., 2014). The control group corresponds to the northern part of Huelva Province, which is a natural park where there is no direct environmental contamination from the ground and the water reservoirs are of good ecological quality (Martín-Machuca et al., 2010). Although, in this area there are some small and isolated vein-type mineralizations, metal mobilization and local impact on soils and water is very restricted. For more information about the geological characteristics and environmental contamination of the Huelva Province the readers are referred to Fernández-Caliani (2008); Galán et al. (2008); Sánchez España (2008); Tornos Arroyo (2008); Madejón et al. (2011); Castillo et al. (2013); Fernández-Caliani et al. (2013), and Rivera et al. (2016).

After surgery, the samples were rinsed with deionized water, let to dry up at room temperature and stored in plastic cups in the dark. First the samples were photographed and visually characterized by color, size, shape, morphology, and amount of calculi. Subsequently, 29 samples that were large enough were divided into subsamples; larger solitary calculi were cut in half and multicalculi samples were subdivided. One half was ground using a pestle agate mortar and a polished epoxy probe (Epofix) was prepared with the other half. The rest of the smaller samples were merely ground. Rough surface samples were collected in case of abundant sample material.

The homogenized and powdered samples were characterized for phase composition using powder X-ray diffraction (XRD) and Fourier Transformed Infra-Red (FTIR) at the analytical facilities of the Instituto Andaluz de Ciencias de la Tierra (IACT). The bulk powder XRD patterns were collected by Cu K_α radiation (lambda = 1.5418 Å) using a PANalytical X'Pert PRO diffractometer equipped with a PIXcel detector operating at 45 kV and 40 mA. The measurements were carried out from 4° to 70° (2θ) with a step size of 0.02°. Automatic-variable anti-scatter slits with a constant irradiated length of 10 mm were used for the incident and diffracted beams. The FTIR spectra were collected using a PerkinElmer Spectrum One FTIR spectrometer equipped with a lithiumtantalate ($LiTaO_3$) detector in nitrogen gas atmosphere. The measurements were carried out in the range from 450 to 4000 cm^{-1} with a resolution of 4 cm^{-1} in absorption mode. A total of 100 scans were collected. Pressed KBr pellets were prepared using 150 mg of KBr and 1 mg of sample in a hydraulic press, and a pure KBr pellet was used to run the background correction.

The polished probes were first observed by an optical microscope for an overall examination and imaging. Subsequently, they were studied by FTIR coupled with a JASCO IRT-7100 microscope using Attenuated Total Reflection (ATR) with a diamond crystal at Centro de Instrumentación Científica (CIC) at the University of Granada. The measurements were run in the range from 650 to 4000 cm^{-1} with a resolution of 4 cm^{-1} in absorption mode, and 300 scans were collected. The spectra were collected using Spectra Manager software. Subsequently, the probes were carbon coated and studied using Environmental Scanning Electron