Correlations Between Morphology and Instrumental Analysis of Human Gallstones - Preliminary Study

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This paper deals with presenting the results obtained after carrying morphology and instrumental investigations over six samples of human biliary stones, as a continuation of our dedicated study in evaluating the physico-chemical properties of these biological samples. The results regarding the composition of samples were correlated with our previous studies in the field of solid human samples (calculi).

Keywords: human biliary calculi, gallstone, composition, thermal analysis, CT investigation

Medical investigations carried for the analysis of biliary disease is wide and mainly divided into two directions: malign and benign pathologies. By far, the most common benign clinical problem is represented by lithogenic processes –i.e. the formation of gallstones, either in bile duct or in the gallbladder [1].

The clinical investigations are generally carried out in a coherent order, starting with Ultrasonography. Other techniques include scintigraphy, magnetic resonance imaging, multidetector computed tomography and positron emission tomography, with increased accuracy in analyzing gallbladder diseases [2].

Ultrasonography is one of the most important investigations since it not requires long time for measurements and radiations over the patient and can be considered of very good accuracy, since in 95% of cases reveal the presence of gallstones. Also, important information over the aspect and structure of the gallbladder wall [1,2] is revealed.

Other imaging techniques, like CT and MRI can reveal the presence of biliary stones, but are considered complementary approaches (second-line ones), mainly used in determining the aspect and size of gallbladder and the existence of inflammatory processes associated with its malfunctionality [2].

Only 74-79% of gallstones are identified in patients with computed tomography (CT) scanning. CT is not a screening tool for uncomplicated cholelithiasis [3].

Three instrumental techniques were used for a rapid analysis of eight biological samples of gallstones, which were intraoperative obtained from six patients from of different ages hospitalized at the Department of Surgery II, First Clinic of Surgery (Timis). The gallstones were different in both aspect and dimensions, but the use of the instrumental techniques revealed that the composition consisted mainly in cholesterol, along with other organic compounds, such as bile pigments and esters. In none of the case, traces of inorganic compounds, such as apatite, aragonite, calcite or phosphates were observed. These preliminary reported results offer a starting point in the analysis of external factors and incidence of cholelithiasis and offer a perspective corroboration of the type of stone with the treating scheme.

Successfully oral litholytic therapy of gallstones require the preliminary evaluation regarding the gallstones composition by both qualitative and quantitative aspects, and literature [4] indicate that CT measurements of stone attenuation score allows a prediction of this composition [5]. Hussaini et al. carried in 1995 a well-documented devoted study regarding the composition of gallbladder stones associated with octreotide, and as well the response to ursodeoxycholic acid oral therapy. Hussaini et al. indicated that stones with attenuation scores below 100 HU indicate cholesterol rich and therefore potentially dissolvable stones, while stones with attenuation score over 100 HU are mainly composed on variable amounts of calcium salts and bile pigments, but as well cholesterol-free stones, composed mainly of brown and black pigments [5].

The main component in the composition of gallstones is cholesterol, as suggested by literature data [5-13].

As previously stated in our studies [9-10], the knowledge of composition of gallstones is of crucial importance in order to determine the lithogenic mechanism, and as well can reveal the incidence at local, regional and/or national level of different types of ethiopatologies.

Since in our previous study we described the FTIR and PXRD data, but as well thermal behavior of cholesterol, in this paper we report solely the result obtained after carrying the analyses of the samples by thermal investigations, correlated with already published results.

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Experimental part

Materials and methods

Gallbladder stones were collected intraoperative at the Department of Surgery II, First Clinic of Surgery (Timisoara, Timis). All the analyses were carried out with patients' agreement. In order to keep the confidentiality of identities, the samples were labeled as Gallbladder stones (numbered 1-6).

All the samples were washed with water–ethanol mixture, dried at 40°C for 24h. Imagistic investigations were carried out at Neuromed Diagnostic Imaging Centre, Timisoara, Romania, on a multidetector computed tomographic (MDCT) (64-slice MDCT system; SOMATOM Sensation, Siemens Medical Solutions, Forchheim, Germany).

All the stones were identically processed; the investigated samples for thermal analysis were taken from the outer layer of the stone, according to imagistic results.

The mass of each stone was determined using an analytical balance (Sartorius), with a precision of four digits.

Thermal investigations of the biologic samples (TG/DTG/HF) were carried out on a Perkin-Elmer DIAMOND device. Samples between 5-8 mg were heated in open aluminum crucibles, from ambient temperature up to 400°C using a heating rate $\beta = 10°C \cdot min^{-1}$, under a dynamic flow of synthetic air ($100\ mL \cdot min^{-1}$). The data are presented in the temperature range 25-400 °C (HF) and 175-400 °C (TG and DTG), for avoiding superimposing of thermal curves and obtain a correct view of the results.

Results and discussions

Visual characterization of samples

The samples were selected from a collection of gallstones, extracted from patients, as presented in table 1.

After extraction of gallstones, the macroscopic analysis regarding the shape and exterior aspect of the samples was carried out (fig. 1). The colour of the sample varies from whitish to dark gray. All samples were further investigated by imagistic technique.

Table 1
ANALYZED GALLSTONES SAMPLES

Sample	Colour
1	whitish
2	light gray
3	light brown
4	light gray
5	dark gray
6	light brown

Imagistic investigations

Since computed tomography (CT) provides highly sensitive measurement of radiodensity, we correlated the results obtained by this imagistic technique with the ones suggested by thermal analysis, in order to determine if the results are comparable. CT measurement of stone density allows a prediction of the composition of radiolucent vs. radiopaque gallstones.

The samples were analyzed by CT imagistic investigation by both lateral (fig. 2) and longitudinal view (fig.3), in order to determine the distribution of possible layers of deposition through lithogenic process. The samples present several variations. The laminar structure of the calculi is clearly visible, as well their formation by depositing concentric layers around some initial condensation nuclei. These initial condensation nuclei are visible in all six imagistic investigations, in the central part of the samples, by both lateral and longitudinal view. It seems that the lithogenic deposition is different: in the case of sample 1, a relatively homogeny deposition is observed, the calculus is not presenting layering, while all other samples are formed by concentric layers while alternating hypodense-hyperdense composition. In the case of calculi 2 and 4, the CT results are also correlated

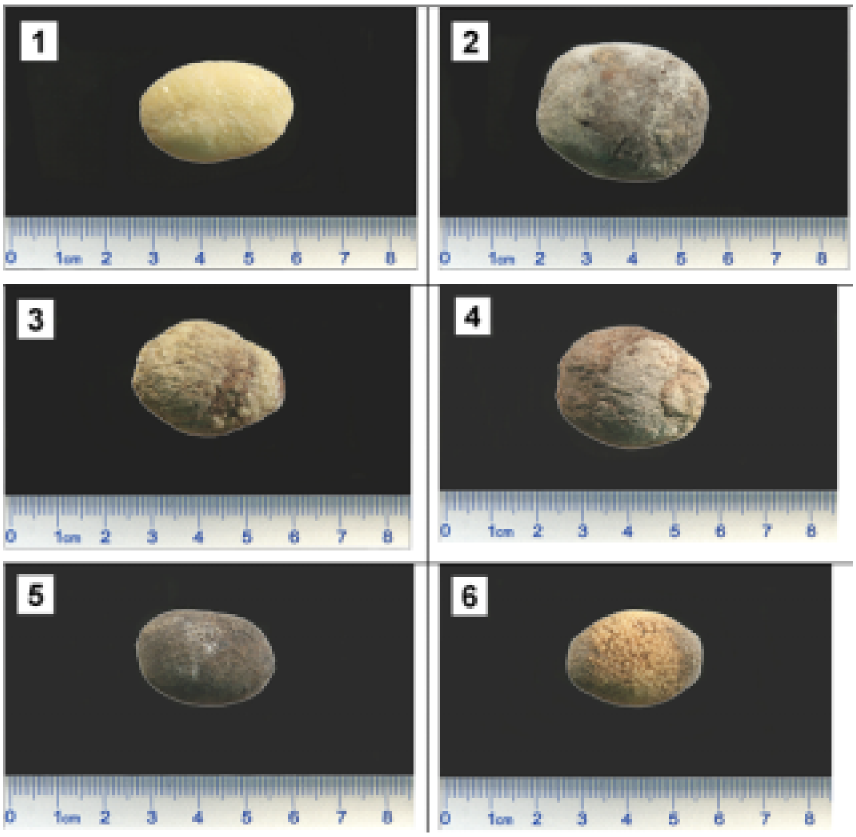

Fig. 1. Shape and exterior view of the analysed GS

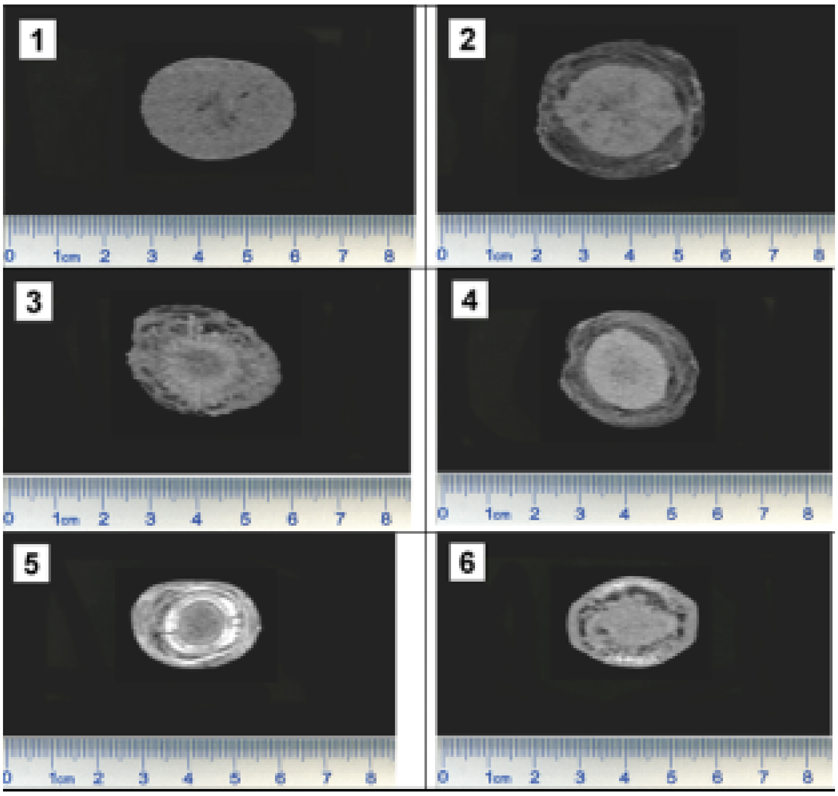

Fig. 2. Imagistic investigation by CT of analysed samples (lateral view)

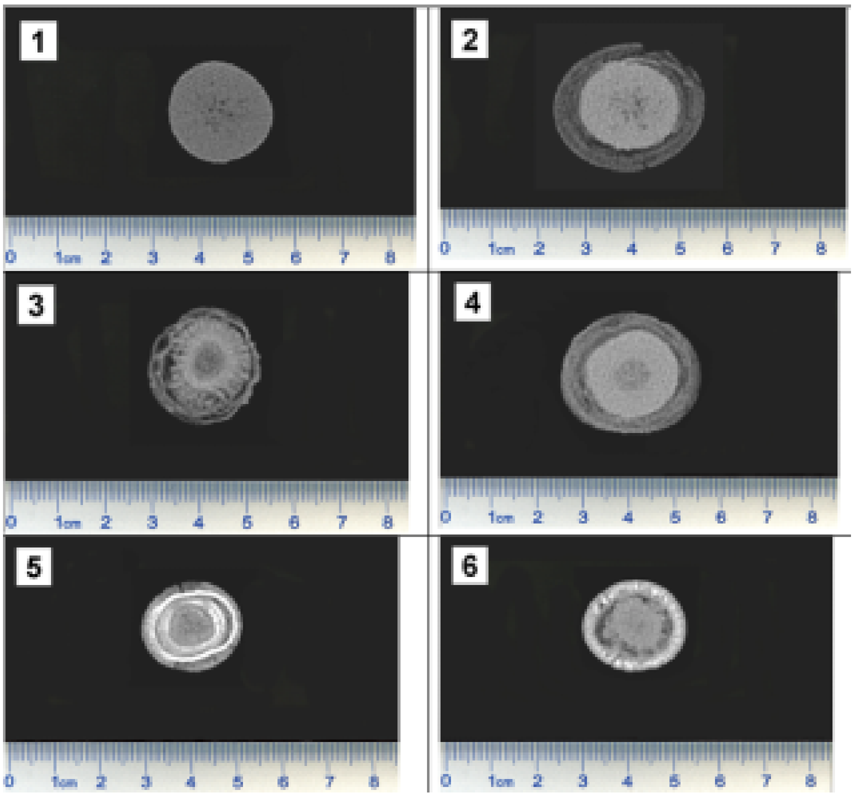

Fig. 3. Imagistic investigation by CT of analyzed samples (longitudinal view)

with their aspect and the sample behaviour during thermal analysis, suggesting a clear separation of two areas.

Along with the dimension of the gallstones (table 1), their volume was determined. For the estimation of their apparent density, the mass/volume ratio was calculated. Also, the values of CT attenuation were correlated with apparent density. In the case of cholesterol-rich stones (samples 1, 5 and 6) the CT attenuation values are in good agreement with the reported literature data that suggest that a value under 100 HU is a clear indication of presence of cholesterol. These samples show an apparent density over 0.93 g/cm^3. Sample 3 has a different distribution of layer, being clearly heterogeneous not only by layers, but also within the layer. The CT results and apparent density indicate an intermediary behavior between pigmented stones and cholesterol-rich stones. As for stones with CT attenuation values over 200 HU (samples 2 and 4), the layered deposition is clearly visible, as well the values for apparent densities falls in the same range, around 0.79 g/cm^3, suggesting more porous structures. The obtained results were correlated with thermal analysis.

Thermal behaviour

The thermolysis of the gallstones was investigated in non-isothermal conditions in air atmosphere and the results

Sample	Length [mm]	Maximum diameter [mm]	Volume [cm³]	CT attenuation mean value [HU]	Mass [g]	Apparent density [g/cm³]
1.	32.15	20.40	7.277	53	7.5178	1.0331
2.	33.90	27.20	11.590	251	9.1454	0.7891
3.	32.80	23.10	8.546	153	7.4865	0.8760
4.	32.15	26.05	10.474	240	8.3262	0.7949
5.	28.80	19.55	5.320	52	5.3933	1.0138
6.	29.15	19.70	5.695	70	5.3267	0.9353

Table 2
THE CT IMAGING INVESTIGATION RESULTS FOR ANALYZED SAMPLES

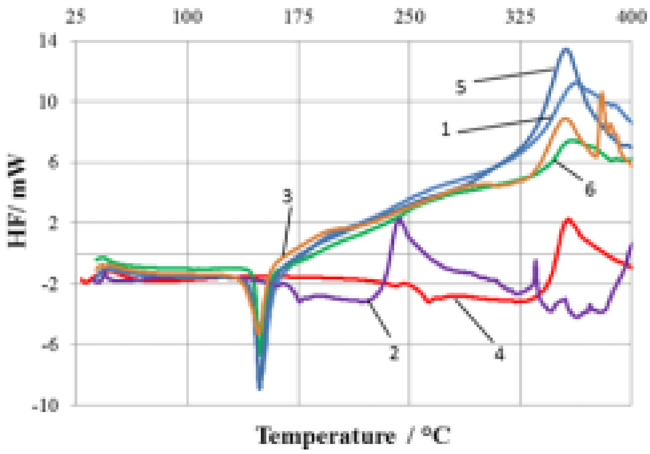

Fig. 4. Heat flow curves for analysed samples

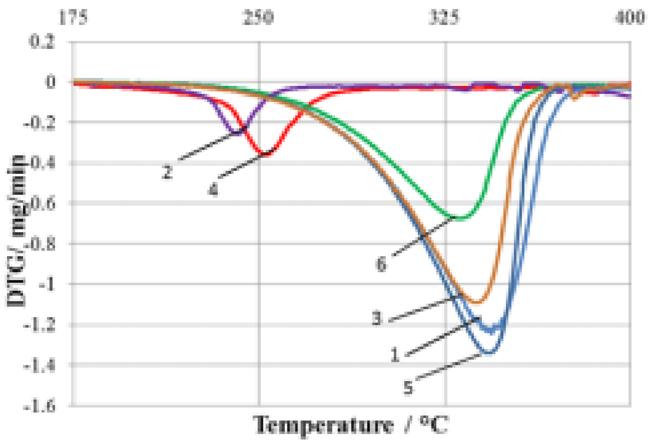

Fig. 5. Mass curves for analysed samples

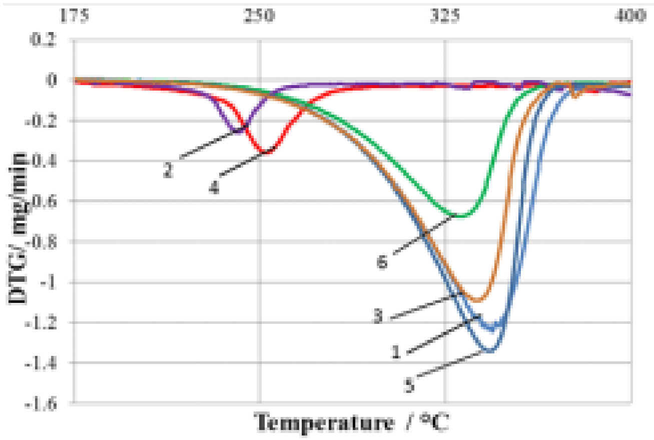

Fig. 6. Mass derivative curves for analysed samples

are presented as superimposed TG, DTG and HF curves (figs. 4-6). According to their thermal profile, a grouping into two classes was observed: cholesterol-rich stones (samples 1, 3, 5, 6) with similar thermoanalytical profiles and mixed stones (pigments and other compounds, like calcium salts, samples 2, 4)

Cholesterol can be identified by the position of melting peak on the HF curves and by the maximum of the DTG peaks.

In literature [14], for cholesterol, the melting point is reported at 148 °C for the anhydrous form and at 146 °C for the cholesterol hydrate, respectively. Before the melting interval, Wada et al. have reported another endothermic peak due to a polymorphic transition (at 40 °C) knowing that the water molecules are bounded in cholesterol. For the samples 1, 3, 5 and 6, the general aspect of the stones and the HF curve allure are similar. These HF curves present a wide and blurred endothermic peak around 40 °C and a prominent melting peak with maximum around 146 °C.

Analyzing the thermogravimetrical curves (mass and mass derivative), for the samples 1, 3, 5 and 6, the mass loss take place in the same temperature range (225-375 °C) with the same value for $\Delta m \approx 90$ %. These mass loss processes are accompanied on the DTG curve by broad and well defined peaks with maximum at $\approx$ 345 °C.

For the samples 2 and 4, the thermoanalytical behavior is different. The melting point for cholesterol is missing. The mass loss appears at lower temperatures, but Äm is comparatively lower (40 %) that in other cases. These samples may contain calcium salts which are stable at high temperatures. Another component of these samples can be biliary pigments which have a complex organic skeleton which can explain the thermal instability of these samples (2 and 4).

According to the literature, the melting interval is broad and the maximum of peak decreases if the presence of impurities in a crystalline organic compound is observed. For these four gallstones (1, 3, 5, 6), all the thermoanalytical curves argue that the samples contain cholesterol in a high purity.

The results are in good agreement with CT investigations, but as well with literature data.

Conclusions

In this paper, we presented a preliminary study regarding the correlation of some instrumental data (thermal analysis) with imagistic analysis (CT) regarding the composition of six human biliary calculi. The obtained results are in good agreement with literature, regarding the imagistic data obtained, but as well with the reported physico-chemical analysis (thermal analysis). However, this study is preliminary, since the complete characterization of composition by quantitative measurements will be realized only after some investigations carried out in homogenous medium, namely after disaggregation of the solid sample and correct estimation of cholesterol and calcium content in the samples.

Acknowledgement: This work was supported by a grant financed by the University of Medicine and Pharmacy Victor Babes Timisoara (Grant PIII-C3-PCFI-2016/2017, acronym STONES to M.S.M, S.O., C.I., I.L. and A.L.).

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Manuscript received: 26.01.2016