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Physico-chemical Properties of Gallstones

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Gallstones found in the gallbladder are classified based on their chemical composition as cholesterol, mixed or pigmented stones. In the present study, the morphology and the chemical composition of gallstones obtained from patients with colelithiasis have been examined. The investigations were carried out using various chemical, spectroscopic and X-radiographic methods. The number of stones obtained from patients with colelithiasis ranged from 1 to 90 and the majority (75%) of these stones was cholesterol or mixed, while the rest (25%) were calcium carbonate, calcium bilirubinate or organic stones. The majority of cholesterol or mixed gallstones analyzed had a layer structure with patches of pigmented material. Multi-stones were small, spherical, and contained fibrous material at their centres. Furthermore, some of these stones were heavily pigmented at their centres. Some stones were long and black with irregular shape and were mainly composed of calcium bilirubinate and had no cholesterol. The majority of gallstones described and analyzed in the target group was cholesterol or mixed in composition. The remaining minority of gallstones was pigmented, which usually have less cholesterol than cholesterol or mixed gallstones.

Key Words: Gallstones, Colelithiasis patients.

INTRODUCTION

Gallstone disease remains an important public health problem because of its extraordinary frequency. Over 20 million Americans have undergone gallbladder surgery or currently have gallstones[1], and more than 700,000 cholecystectomies are performed annually[2, 3]. Risk factors for gallstone disease are obesity, rapid weight loss, smoking, low physical activity, low intake of dietary fibre and high intake of carbohydrates[4]. Other risk factors include cholesterol-lowering drugs that increase the amount of cholesterol secreted in the bile thereby increasing supersaturation and precipitation[5–7].

Gallbladder stones are classified based on their chemical composition as cholesterol, pigmented or mixed stones[2]. Approximately 90% of gallstones are cholesterol, consisting of more than 50% cholesterol or mixed, containing

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between 20–50% cholesterol. Gallstones that have less than 20% cholesterol are considered pigmented stones[2]. The principal mechanism triggering the formation of gallstones is supersaturation with ingredients in the bile exceeding their maximum solubilities[2, 3]. Other factors contributing to gallstone formation are nucleation factors, bile stasis within the gallbladder and calcium in bile.

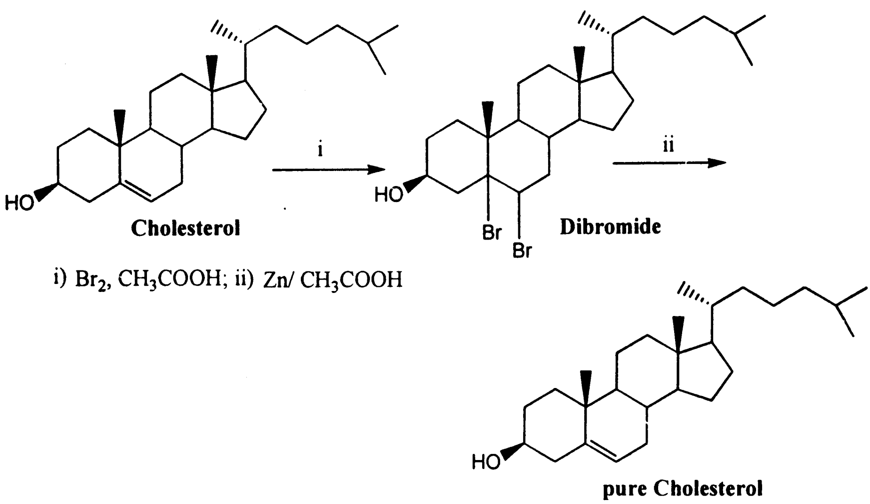
In the present study we report the morphological and chemical composition of gallstones isolated from patients with gallstone disease.

EXPERIMENTAL

General: Fifteen stones obtained from patients with colelithiasis, whose gallbladders were surgically or laparoscopically removed in the Surgical Department of Al-Bashir Hospital, Amman, Jordan were analyzed in detail.

Melting point of cholesterol was determined on a Thomas-Hoover capillary melting point apparatus and was uncorrected. Infrared spectra were recorded on a Nicolet-Impact 410 FT-IR spectrometer. ^{1}H-NMR spectra were recorded using 200 MHz Brucker. The control cholesterol 95 per cent (Acros) was recrystallized from acetone before usage.

Cholesterol and other components of gallstones were analyzed by wet chemical, spectroscopic and X-ray diffraction methods.



Scheme 1: Purification of cholesterol isolated from cholesterol stones by bromination-debromination.

Wet chemical method: 1 g of each analyzed stone was grounded and completely dissolved in 8 mL of diethyl ether that gave a brownish-yellow solution. The brownish-yellow solution, which is the metabolic oxidation product of hemoglobin (bilirubin), was filtered. An equal volume of methanol was added to the filtrate and 0.1 g of charcoal was also added to remove the greenish-yellow impurities. Evaporation of the solvents *in vacuo* gave a solid material that was recrystallized from cold methanol to yield crude cholesterol. To ensure the proper calculations of pure cholesterol content of the analyzed gallstones, the crude cholesterol was subjected to bromination and debromination[8] **(Scheme 1).**

To determine the amount of the crystalline components that was present in the analyzed stones, 0.5 g of each analyzed stone was dissolved in benzene to dissolve the cholesterol. The residue, which was insoluble in benzene, contained calcium carbonate and calcium hydrogen phosphate that was examined by X-ray diffraction. When applicable, Fourier Transform-Infrared Spectroscopy (FT-IR) was used to identify calcium bilirubinate or to confirm its absence.

Spectroscopy and X-radiography: Pure cholesterol obtained form the wet method was subjected to analysis by FT-IR, according to a modified systematic scheme devised by Oliver and Sweet,[9] proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and X-radiography methods.

RESULTS AND DISCUSSION

Table-1 shows the characteristics of the analyzed gallstones. Patients 1, 2, 14 and 15 each had one large cholesterol solitaire stone. These stones were approximately similar in shape and size. Stones from patients 1 and 14 had smooth, dark grey-green surfaces and stones from patients 2 and 15 had knobby, cream-coloured surfaces. The outer, dark-coloured layers of stones from patients 1 and 14 were 0.4 and 0.5 mm thick, respectively.

TABLE-1
CHARACTERISTICS AND STRUCTURE OF GALLSTONES

Patient No.	Stone size (cm)	No. of stones	Cholesterol (%)	Stone structure
1	1.4	1	92	$CaCO_3$ shell. Interior is
14	1.5	solitaire	95	layered like a tree trunk
2	1.1	1	95	Large cholesterol crystals
15	1.8	solitaire	97	lying along stone radii
3	0.2–0.9	36	97	
4	0.3–0.7	56	98	Heavily pigmented centre.
7	0.4–1.2	90	99	No layer structure.
13	0.5–0.9	78	99	
5	0.7–1.6	8	96	Radial cholesterol crystals, columns of $CaCO_3$.
6	0.3–0.5	31	99	Radial cholesterol crystals, heavily pigmented centre
8	1.2	1	0	Black $CaCO_3$ stone.
9	4.0 × 1.0	1	0	Black calcium bilirubinate
12	3.5 × 0.8	1	0	stone.
10	0.8	18	0	Black organic stone,
11	0.6	23	0	m.p. > 320°C.

They were easily separated from the rest of the stones and consisted mostly of calcium carbonate. On the other hand, the outer layers of stones from patients 2 and 15 were composed of cholesterol. The internal structures of these four stones also varied. Stones from patients 1 and 14 had a structure similar to that of a tree trunk built up of many layers of cholesterol containing patches of pigmented material. The thickness of each layer was 0.01–0.02 mm and such a layer corresponded to approximately 5 mg of cholesterol. The pigment was present as spots in each layer and some layers were more heavily pigmented than others. The centre of stones from patients 1 and 14 were poorly defined and consisted of three adjacent heavily pigmented areas. On the contrary, stones from patients 2 and 15 had a well-defined pigmented center with large cholesterol crystals radiating out from the centre. The shiny cleavage faces of the crystals could be easily seen in the broken stones.

Multiple deposits of cholesterol stones were also common. Such stones, from patients 3, 4, 5, 6, 7 and 13, were very small and spherical. Their centers were heavily pigmented and crystals of cholesterol were radiating out from the center. These stones were similar to the solitaire stones from patients 2 and 15, but they were much smaller, more numerous and more heavily pigmented.

Stones from patient 6 were also structurally very similar to the cholesterol solitaire stones form patients 2 and 15 and again the calcium carbonate was present almost entirely as vaterite. Furthermore, micro-X-radiographs of thin sections of gallstone of patient 5 showed that the calcium carbonate was distributed in discontinous rings around the center. When the stone was treated with benzene to remove the cholesterol, the structure of the calcium carbonate deposits could be seen as columns radiating towards the centre. However, layer formation was also still visible and the layers of deposit seemed to be about 0.01 mm wide. It was shown by X-ray diffraction that the columns of calcium carbonate crystals had not grown in any preferred orientation.

The infrared spectrum (Fig. 1) indicates that these stones contain cholesterol, calcium bilirubinate and calcium carbonate.

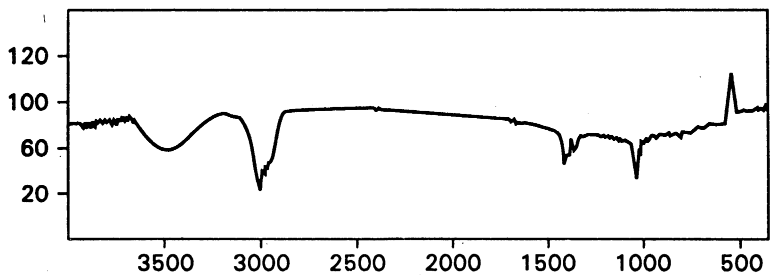


Fig. 1 FT-IR spectrum for cholesterol stones after bromination-debromination

Stone from patient 8 was large, hard, heavily pigmented and contained mostly calcite and some vaterite. Interestingly, cholesterol was not found in this stone. When viewed under the optical microscope, the stone had a porous structure and the crushed stone consisted of yellowish crystals and few darker reddish brown crystals.

Stones from patients 9 and 12 were long, black and irregularly shaped. Their infrared spectrum indicated that they were calcium bilirubinate stones.

Stone from patient 11 was small and had a shiny black colour. It contained neither cholesterol nor calcium carbonate. When heated, it burnt and left only a small black residue. This stone was composed mainly of organic material, probably, a derivative of an amino acid, such as taurine and a pigment material. FT-IR analysis showed that this stone did not contain any detectable quantity of calcium bilirubinate.

In this study, we report the morphological and chemical composition of gallstones isolated from Jordanian patients with gallstone disease. The number of stones per patient varied from 1 to 90. The majority of stones was mainly composed of cholesterol and contained pigmented material, especially at the centre. The results showed that 75% of patients had cholesterol stones; the rest had calcium carbonate, calcium bilirubinate or organic stones.

The FT-IR spectra of cholesterol obtained from gallstones, showed the appearance of carbon-carbon double bond at 1640 cm^{-1} (Fig. 1) and the ^{1}H-NMR spectra demonstrated the peak at 5.3 ppm which is characteristic for the double bond protons which disappeared after bromination and a new peak appeared at 3.0 ppm that is characteristic of the proton on the carbon that has the bromine atom (Fig. 2).

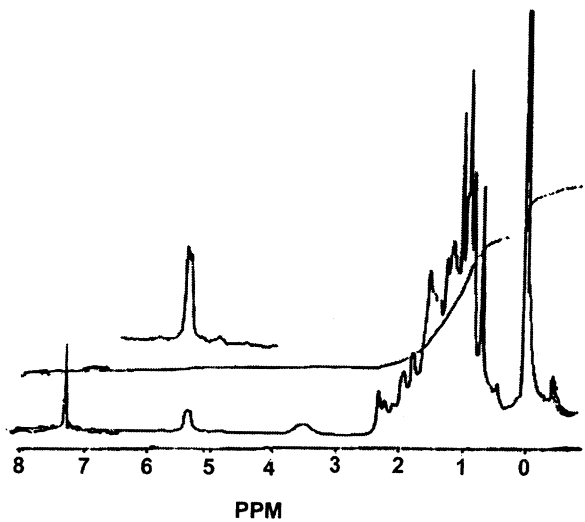


Fig. 2 ^{1}H-NMR spectrum for pure cholesterol isolated from cholesterol stones

It may be inferred that a pigmented calcium carbonate stone must be the result of a different physiological disorder from that which produced a cholesterol stone. Additionally, the calcium carbonate was accompanied by pigmented material and the crystals of calcium carbonate were always coloured. Normal bile contains calcium and bicarbonate ions, but precipitation of calcium carbonate could be brought about by a change in the pH of the bile, which is normally controlled by the re-absorption of the bicarbonate ions and water through the walls of the gallbladder. Any factor, which disturbs this bicarbonate re-absorption, could result in the formation of solid calcium carbonate[10]. The precipitation of calcium carbonate does not, however, explain the formation of a stone. It would be expected that the precipitate would simply form a milky suspension, which would be expelled from the gallbladder with bile in the normal way. If, however, the precipitates were trapped in some biliary debris or mucosubstances, then stone formation might be able to take place as reported by Bouchier[11]. Alternatively, if calcium carbonate precipitation occurred over a long period of time, then the stone might be able to grow, despite the filling and emptying of the gallbladder, in the same way that calcium carbonate deposits occur in a kettle.

Bilirubin is normally present in the bile as bilirubin diglucuronide[12–14]. If, for some reason, this compound breaks down to give bilirubin and a glycoside residue, then the free bilirubin will always react with the calcium ions present to form a precipitate of calcium bilirubinate, which was proven by FT-IR (Fig. 1). It is believed that bacterial infection can be responsible for such bilirubin diglucuronide breakdown[15] and hence for some calcium bilirubinate stones. It has been shown, in rabbits, that the gallbladder epithelial cells secrete increased amounts of mucous substances just before gallstones start to form[16].

The present study has indicated that pigmented material is always present at the centres of stones and that the stone centres were often a mass of small crystals and flaky pigmented material trapped in strands of fibres. This study indicates that nucleation occurs on biliary debris or mucous substances in some gallstones. Those stones, which consist mostly of cholesterol, can have either a layer structure, which has obviously built up step by step around a small central area or can have a large pigmented central area which has no layer structure but is surrounded by a coating consisting of thin layers of cholesterol. The multi-stone deposits fall in this latter category and the stones usually contain fibrous material at their centres.

In some cholesterol stones containing calcium carbonate, the calcium carbonate is deposited very unevenly, indicating that for some periods during stone growth barely any calcium carbonate is deposited. Calcium carbonate is often in the form of the rare vaterite and can be present as columns of small crystals without any preferred orientation. The cholesterol in such stones is most likely to be in large orientated flakes radiating from the centre. Calcium carbonate occurs in gallstones in all three of its known crystalline forms and is always associated with pigmented material, although pigmented material can occur without calcium carbonate being present.

Conclusion

Gallstones isolated from patients vary in their number and mainly are composed of cholesterol with pigmented material at the centre. Other types of stones including calcium carbonate, calcium bilirubinate and organic stones were also found in 25 per cent of the cases.

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