Structural analysis of gallstones with thin-section petrographic microscopy: A study of 100 gallstones from Taiwanese patients

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Cholecystolithiasis is a common disease, making cholecystectomy a commonly performed surgical procedure. The gross appearance of gallstones differs from case to case. To classify gallstones on the basis of their structure, we randomly collected gallstones from 100 (64 of them women) of 3289 patients who underwent cholecystectomy at our hospital. The stones were grossly classified into five major types: pure-cholesterol stones, combination stones, mixed stones, black stones, and calcium bilirubinate stones. We then used thin-section petrographic microscopic study (TSPMS) to inspect each stone under a polarizing microscope. Final classification of the stone depended on TSPMS findings. Of the 100 patients, 35 had pure-cholesterol stones, 12 had combination stones, 17 had mixed stones, 25 had black stones, and 8 had calcium bilirubinate stones; the stones from 3 patients could not be classified on TSPMS. Accurate structural classification of gallstones could be made by gross inspection with confirmation by TSPMS, a useful method of classifying gallstones. (J Lab Clin Med 2002;140:387-90)

Abbreviations: LC = laparoscopic cholecystectomy; TSPMS = thin-section petrographic microscopic study

Cholecystolithiasis is a common disease, in Taiwan and in many other countries. Most patients with symptomatic cholecystolithiasis require surgical treatment; thus cholecystectomy is a commonly performed procedure. In the Department of General and Gastrointestinal Surgery of Cathay General Hospital, 3289 LCs have been performed since we introduced the procedure to Taiwan in 1990.[1] As might be expected, the gross appearance of recovered gallstones — size, shape, color, hardness, and number — has differed from case to case. Macroscopic classification of gallstones as proposed by the Japanese Society of Gastroenterology is based on the presence of characteristic structures on the cut surface.[2] According to this classification, gallstones can be broadly divided into three types: cholesterol gallstones, pigment gallstones, and rare gallstones. This classification is popular in Japan because of its clinical applicability.[2] In this study we sought to classify gallstones from Taiwanese patients on the basis of structure by conducting a gross inspection, recording the characteristics of each type, and further studying the stones with the use of the TSPMS method. Because of the fragile nature of some gallstones, it can be difficult to generated sections thin enough for microscopic study by conventional urological methods. We tried to apply TSPMS to solve this problem, after which we analyzed the results. To the best of our knowledge, this method of gallstone classification has not been reported before.

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METHODS

Collection. We randomly selected 100 gallstones (64 of them from female subjects) from 3289 patients (64% female) who underwent LC for cholecystolithiasis at our hospital. Stones from the 1183 male patients were numbered M1

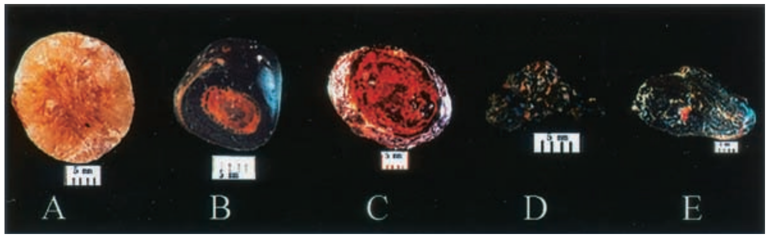

Fig 1. Gross appearance of the cut surfaces of the five major types of gallstones. **A**, Cholesterol stone. The cut surface is smooth, glossy, and a semitranslucent brown. **B**, Combination stone. Its structure is composed of laminae of light-brown cholesterol crystal and dark-brown calcium bilirubinate. **C**, Mixed stone. Layers of cholesterol crystal and calcium bilirubinate are intermingled, with some clefts evident. **D**, Black stone. Mainly black in color, irregular in shape, and amorphous in structure. **E**, Calcium bilirubinate stone. All laminated layers are dark brown.

through M1183, and those of the 2106 women were numbered F1 through F2106. Next we used Microsoft Excel, embedded a formula as "round (rand()*1182 + 1,0)," and pressed the F9 key 36 times for the 36 male patients. The same procedure was applied for the female subjects to yield 64 numbers, only the formula was changed to "round (rand()*2105 + 1,0)." The subjects ranged in age from 24 to 81 years (mean 53.7).

Gross inspection. We took pictures of individual sample stones, cut each stone at the equator with a saw and then polished the cut surface with sandpaper. After the cut surfaces of all stones were cleaned with a soft brush, the stones were divided on the basis of the findings of gross inspection of the cut surfaces into five major categories. The categories, based on shape, color, hardness, and structure, were as follows: pure-cholesterol stones, combination stones, black stones, and calcium bilirubinate stones.

TSPMS. In preparing for TSPMS, we used an impregnation machine (Epovac; Struers, Anahiem, Calif) to impregnate each stone with a fixative (Epofix; Struers). After impregnation, a thin slice of the stone was made with a cutting/grinding machine (Discoplan; Struers); this slice was then fixed on a glass slide. The prepared slide was observed under a polarizing light microscope (BH-2; Olympus, Tokyo, Japan). Photographs of each specimen were taken with an exposure-control unit (AD system; Olympus, Tokyo, Japan). The classification of gallstones made on the basis of gross inspection was then confirmed on TSPMS.

RESULTS

Cholesterol stones were of three subtypes: pure-cholesterol stones, combination stones, and mixed stones. Pigment stones were of two subtypes: black stones and calcium bilirubinate stones. These five types were the focus of our study. No rare stones were identified.

The gross appearances of the cut surfaces of the five major types of gallstone are shown in Figure 1. Pure-cholesterol stones are white or light brown, and they are round. The number of stones in each case varied from solitary to multiple. The shiny appearance of the cut surface of the pure-cholesterol stone radiated out from the center of the stone (Fig 1, *A*). Combination stones were composed of both cholesterol and calcium bilirubinate or black stone with clear boundaries between each component apparent on the cut surface (Fig 1, *B*). Mixed stones were composed of cholesterol and calcium bilirubinate or black stone with indistinct boundaries between components (Fig 1, *C*). Black stones were pure black in color, irregular in shape and structure, comparatively small, and usually present in multiples (Fig 1, *D*). Calcium bilirubinate stones were dark brown, soft and fragile in consistency, and light in weight (Fig 1, *E*). Among the 100 samples, we unable to make a definitive classification on the basis of gross inspection of the cut surface in 26 instances.

Figure 2 shows gross the appearances of thin sections of the five major types of gallstone. Cholesterol stones were mainly a semitranslucent brown, with areas dyed dark brown by bile (Fig 2, *A*). Combination stones had dark-brown calcium bilirubinate, sandwiched by outer and inner layers of light-brown cholesterol crystal (Fig 2, *B*). Mixed stones were composed of layers of cholesterol crystal and calcium bilirubinate, irregularly interlaced; clefts were noted between two or three components (Fig 2, *C*). Black stones — which were mainly black, irregular in shape, and amorphous in structure — are shown in Figure 2, *D*. We show a calcium bilirubinate stone with the whole stone made up of dark-brown laminated layers (Fig 2, *E*). Inspection of gross appearance on the thin section helped us classify half of the stones that could not be classified on gross inspection of the cut surface.

Thin sections of the five major types of stones under polarized light microscopy are shown in Figure 3. Microscopically, the thin section of cholesterol revealed radial cholesterol crystal structures. Under the microscope, the crystal exhibited the "play of color" phenomenon (Fig 3, *A*), in which rotation of the sample under the polarized light microscope changed the pattern of the colors. Under the microscope, the thin sec-

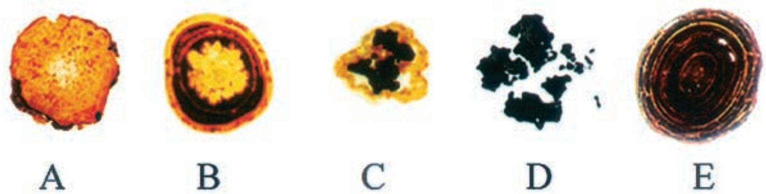

Fig 2. Gross appearance of thin sections of the five major types of gallstones. **A**, Cholesterol stone. Mainly a semitranslucent brown with some areas stained a dark brown by bile. **B**, Combination stone. A dark-brown calcium bilirubinate layer is sandwiched by outer and inner light-brown cholesterol-crystal layers. **C**, Mixed stone. Layers of cholesterol crystal and calcium bilirubinate are irregularly intermingled, and occasional clefts are evident. **D**, Black stone. Mainly black in color, irregular in shape, and amorphous in structure. **E**, Calcium bilirubinate stone. The whole stone is composed of dark-brown laminated layers.

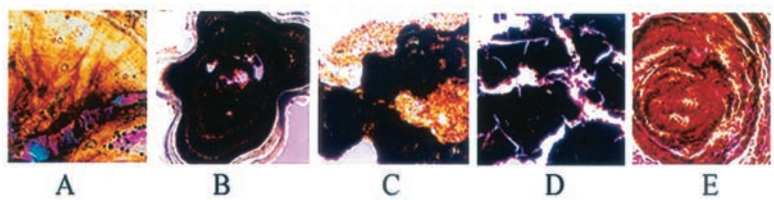

Fig 3. Polarized microscopic appearance of thin sections of the five major types of gallstones (original magnification × 40). **A**, Cholesterol stone. The cholesterol crystal is arranged in a radiating pattern and exhibits the "play of color" phenomenon. **B**, Combination stone. The cholesterol-crystal layers (*outer layers*) are transparent, and the calcium bilirubinate layers (*inner layers*) are nontransparent. **C**, Mixed stone. Cholesterol crystal (*brown*) is irregularly scattered in calcium bilirubinate (*black*). **D**, Black stone. Irregular structure with randomly scattered pieces of broken black pigment. **E**, Calcium bilirubinate stone; structure resembles the growth rings of a tree.

tion of combination stone showed that the boundary between the light-brown cholesterol crystal and dark-brown calcium bilirubinate was distinct (Fig 3, *B*). In contrast, components of the mixed stone were randomly intermingled and displayed distinct characteristics (Fig 3, *C*). We noted no fixed structure in the black stones, with pieces of broken black pigment scattered randomly (Fig 3, *D*). The arrangement of layers on the cut surface of a calcium bilirubinate stone resembled the growth rings of a tree (Fig 3, *E*).

Of the 100 patients, 64 had cholesterol stones and 33 had pigment stones; 3 patients' stones could not be classified on TSPMS. Most cholesterol stones were pure cholesterol (35 of 64, 55%), followed by mixed stones (17, 27%) and combination stones (12, 19%). Black stones and calcium bilirubinate stones accounted for 69% of (25 of 33) and 31% (8) of pigment stones, respectively (Table I). Eleven patients each had a single stone, and 89 patients had multiple stone.

Table I. Sex distribution of the different types of gallstones

		Patients	
Type of stone	No. of stones	Male	Female
Pure-cholesterol stone	35	9	26
Combination stone	12	4	8
Mixed stone	17	9	8
Black pigment stone	25	10	15
Calcium bilirubinate	8	3	5
Unclassified	3	1	2
TOTAL	100	36	64

DISCUSSION

Gallstones can be analyzed and classified with various techniques, such as gross inspection, polarized light microscopy, and infrared absorption spectroscopy.[2,3] Gross inspection depends in large part on the experience of the examiner, making its accuracy somewhat variable. Polarized light microscopy can be used to analyze only a small part of a stone, which may not represent the characteristics of the stone as a whole. Infrared absorption spectroscopy requires expensive equipment, and sampling of the stone is time-consuming. The TSPMS technique used in this study requires only relatively simple equipment and inexpensive sample preparation. Furthermore, the learning curve for achieving accurate classification is short.

It is believed that cholesterol stones tend to occur more frequently in urban populations and pigment stones tend to occur more frequently in rural populations because of dietary differences. Pure-cholesterol stones occur in patient whose cholesterol is oversaturated and whose apoprotein I (of high-density lipoprotein) is too low to prolong the nucleation time and prevent stone formation. Combination stones, those comprising cholesterol and pigment, may indicate a co-morbidity of gallstones and cholangitis.[4]

In our study, patients with pure-cholesterol stones (more than 90% of the cut surface area composed of cholesterol crystal) accounted for 35% of the study group. In a study of West African subjects, Darko et al[4] reported that 34% of their patients had stones of more than 70% of cholesterol by weight.[5] Aziz and Wandwi[6] reported that the ratio of cholesterol to pigment stones, as ascertained through macroscopic classification, was 2:1 in Tanzanian subjects. It would appear that in both developed countries and underdeveloped countries, cholesterol is the main constituent of gallstones. Additionally, the finding that 89% of our patients had more than one stone is consistent with the 90% figure reported by Aziz and Wanwi.[7]

In a study of Korean subjects, Kim found cholesterol stones, black-pigment stones, and brown pigment stones in 58.1%, 25.2%, and 12.1% of patients, respectively.[8] Our patients had cholesterol stones, black-pigment stones, and brown-pigment stones (calcium bilirubinate) in proportions of 64%, 25%, and 8%, respectively. The order of frequency was the same, and the ratios were quite similar.

Cetta noted that black-pigment stones can coexist with cholesterol stones in a single gallbladder and reported an incidence of this coexistence of 1.06% (13 cases in a surgical series of 1226 patients with gallbladder stones).[9] Because we only sectioned the biggest stones in cases with multiple stones, it is possible that we missed such situations in our study.

Rare stones such as calcium carbonate stones, calcium triglyceride stones, and other combination stones, as reported by Sato et al,[2] were not found in our study. This may have been a consequence of the small sample size in our study.

Inspection of the gross appearance of the cut surface of stones only allowed us to distinguish pure-cholesterol stones from the other types of stones with confidence in two-thirds of the stones; the remaining third of the stones' classifications needed further confirmation. After the thin sections of the stone were observed with the naked eye, components of the stones became more distinct, and nearly 80% of the stones could be classified. Finally, by observing the stones using polarized light microscopy, we were able to classify 97% of the stones. Without thin sections, the detail structure of the crystals can not be seen by the naked eye. After thin-sectioning, some typical stone such as pure-cholesterol stones can be distinguished with the naked eye, but nontranslucent stones can only be seen on a thin slice with a microscope under illumination. The remaining three unclassified stones required x-ray spectrometry or infrared absorption spectroscopy[3] for analysis of their constituent components.

Stone types and compositions have been reported to be related to various symptoms, meaning that gallstone disease is not a distinct clinicopathological entity but a heterogeneous group of different pathogeneses, each of which has its own clinical manifestation, biological behavior, and treatment requirements.[9]

This study revealed TSPMS as a useful technique to improve the accuracy of stone classification. Through correlation of the results of stone analysis with preoperative image studies, the treatment of cholelithiasis may be improved.

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