RESEARCH ARTICLE

The Discrepancy between Gross Features and Chemical Compositions in Gallbladder Stone: A Descriptive Single Center Study in the United Arab Emirates

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Abstract:
Background and Study Aim:
Gallbladder stone (GBS) is a common gastrointestinal disease that is the primary indication for cholecystectomy. The present study was conducted to describe the chemical composition of gallstones in a tertiary referral hospital in the United Arab Emirates.

Materials and Methods:
Patients diagnosed with GBS and who underwent cholecystectomy due to symptomatic GBS and cholecystitis in Sheikh Khalifa Specialty Hospital were enrolled in this study. After cholecystectomy, all stone specimens were classified according to their gross findings into 4 groups, namely black pigmented stones (BLPS), brown pigmented stones (BRPS), mixed cholesterol stones, and cholesterol stones (CLS). Quantitative analysis using Fourier transform infrared spectroscopy was then performed to define the stones’ chemical constituents. They were reclassified into two groups as CLS (cholesterol ≥ 60%) and pigmented stones (PGS, cholesterol ≤ 59%) based on gallstone composition analysis.

Results:
A total of 237 stones were divided into four groups based on their gross findings; cholesterol stones (32.0%), mixed cholesterol (29.2%), black pigmented (26.4%), and brown pigmented (12.3%). After chemical composition analysis, they were resorted into the two following groups according to their cholesterol proportions: pigmented (28.3%) and cholesterol (71.7%). There were significant statistical mean age differences between the pigmented and cholesterol stone groups (58.5±19.8 vs. 34.4±11.0, p < 0.01).

Conclusion:
This descriptive study showed the hospital-based clinical incidence of GBS and suggested that there might be a discrepancy in stone classification based on gross findings and chemical compositions. Moreover, pigmented stones are more likely to be present in older patients than cholesterol stones.

Keywords: Gallbladder stone, Cholecystectomy, Gallstone analysis, Black pigmented stone, Brown pigmented stone, Mixed cholesterol stone, Cholesterol stone, Age, Female.

1. INTRODUCTION

Gallbladder Stone (GBS) is a prevalent gastrointestinal disease that is present in 10–15% of adults worldwide [1], and as such, it is considered a frequent cause of hospital admission with a high economic burden [2, 3]. Traditionally, gallstones have been classified as Cholesterol Stones (CLS), Brown Pigmented Stones (BRPS), and Black-Pigmented Stones (BLPS) according to their morphological features and composition. Each group of stones has been known to have different pathogenesis [4]. Many previous studies have shown that geographic and ethnic factors play a great role in
determining the gallstone characteristics and its regional prevalence [1].

Therefore, the demand for region-specific environmental and demographic predictive factors for gallstone diseases has increased in the United Arab Emirates (UAE). However, there are little data about the prevalence of gallstone disease, common gallstone types with composition profile, or epidemiologic studies in this region. The aim of this study was to describe the characteristics of patients with symptomatic gallstone diseases based on their stone composition analysis results and to suggest future epidemiologic study plans.

2. METHODS

We performed a retrospective cross-sectional study by reviewing medical records at a single institute. Patients diagnosed with symptomatic GBS who underwent a cholecystectomy in the Sheikh Khalifa Specialty Hospital Ras Al Khaimah, UAE, between 2016 and 2018 were enrolled in this study. The diagnostic criteria for symptomatic GBS included the typical clinical symptoms (e.g., abdominal pain [right quadrant or epigastric pain] with or without radiating pain) and positive imaging findings for gallstones or cholecystitis (ultrasound, computed tomography, or magnetic resonance imaging) and were confirmed by postoperative pathological results. All the results were reviewed by an expert panel composed of a clinician, surgeon, radiologist, and pathologist. Of the 150 cholecystectomy cases, 46 were excluded because the gallstone composition analysis results were not available (no stone, tiny sludge was not available for analyses, and gallbladder polyp cases).

After cholecystectomy, gallstone specimens were cleaned with deionised water and photographs were taken on a green background. They were then dried, stored in a plastic container, and sent to a laboratory for composition analysis. The stones were ground thoroughly and mixed with KBr buffer at a ratio of 1:150 to form a disc. The main crystal components were analysed using a Bruker Fourier-transform infrared spectrometer (TENSOR27, Germany) with a frequency range of 400-4000 cm$^{-1}$ and a resolution of 4 cm$^{-1}$. Control substances were obtained from Sigma Chemical Company (St. Louis, MO, USA). Each specimen composition was determined after a comparison of the gallstones with the standard control spectra [5].

The specimens were classified into four different gross morphologic groups, according to the surgeons’ inspection (BLPS, BRPS, mixed cholesterol stones, MCS and CLS, (Fig. 1). After examining the cholesterol distribution of stones of all four groups, we reclassified the stones into two groups (pigmented stones, PGS and CLS) based on the proportion of cholesterol composition. We defined CLS as a stone that was composed of more than 60%, while those with less than 59% were classified as PGS.

To compare the relationships with the factors that have been known as precursors of the two gallstone subgroups, we also collected relevant data regarding demographics (age, sex, body mass index (BMI), gynecologic history, education level, ABO blood types), behavioral characteristics (smoking/alcohol consumption), history of chronic diseases (Diabetes Mellitus (DM), Hypertension (HTN), hyperlipidemia, cerebrovascular disease, coronary artery disease, and fatty liver disease), laboratory results (serum cholesterol level, chronic hepatitis B and C, Helicobacter infection), and lithologic features (stone size and number) from the patients’ medical records. Overweight was defined as a BMI of 25 – 29.9 kg/m$^2$, while a BMI of 30 kg/m$^2$ or higher was considered obese [6]. In order to define the underlying comorbidities of DM and hyperlipidaemia, we referred to the guidelines of the National Cholesterol Education Program Adult Treatment Panel III [7]. Fatty liver disease was diagnosed by increased liver echogenicity compared to that of kidney parenchyma on the same side [8].

Fig. (1). Various types of gallstones classified morphologically in terms of colour, texture, shape, and size. (A) cholesterol stones; (B) brown-pigmented stones; (C) black-pigmented stones; (D & E) mixed cholesterol stones; (F) unclassified crystals.
For the statistical analyses, all numeric variables were presented as mean with standard deviation and median with minimum and maximum values. Nominal variables were depicted as proportions. We compared the statistical differences between the mean values of each stone group using a Student’s t-test and the chi-square test. For the two-sided tests, a p-value < 0.05 was considered statistically significant. To estimate the correlation between the risk factors and outcome values, all the statistical significances were assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs) in multivariate analysis by using the Logistic Regression Model. All statistical analyses were performed using R version 3.0.2 (The R Foundation of Statistical Computing, Seoul, South Korea).

3. RESULTS

A total of 106 cases were included for gallstone composition analyses from the 150 symptomatic gallstone patients confirmed by pathologic investigation after cholecystectomy. The study population comprised mostly UAE nationals (89.7%) and included some expats from the Middle East (5.7%) and North Africa (0.7%). The most common gallstone type based on gross morphologic finding investigations by surgeons was CLS (32.0%), followed by MCS (29.2%), BLPS (26.4%), and BRPS (12.3%) (Fig. 1). The three major components found in the gallstone analyses were Cholesterol (CL), Calcium Bilirubinate (CB), and Calcium Carbonate (CC). Additionally, some trace crystals, including calcium oxalate crystals and mixed bile pigment crystals, were also detected. Table 1 shows the distributions of the three major components (CL, CB, and CC) in each of the gross morphologic groups. We found that the three morphologic gallstone groups, CLS, MCS, and BRPS, had higher levels of CL than BLPS. However, there was no significant difference in terms of CL mean proportion (Fig. 2), Boxplot chol-st.gr.

According to our definition, five cases (17.9%), defined as initially BLPS based on gross morphologic findings before composition analysis, were reassigned to the CLS group. In addition, 11 cases (84.6%) of BRPS and 27 (87.1%) of MCS were also reclassified into the CLS group. Meanwhile, only one case of CLS was reassigned to the PGS group. After the re-classification based on 60% CL content, the histogram of the distribution of stones with the same cholesterol proportion, which showed wide overlapping areas across the three groups except for the BLPS group (Fig. 3A), became clearer to present the different contents of CL between the PGS and CLS groups (Fig. 3B).

Finally, we divided all stone specimens into two groups, PGS (30, 28.3%) and CLS (76, 71.7%) group. After analyzing the composition of the gallstones, we compared the demographical, behavioural, and clinical characteristics between the two groups (Table 2). No differences were discovered between the two groups in terms of BMI, parity, obesity, education level, ABO blood types, smoking and alcohol consumption, DM, fatty liver disease, serum cholesterol level, chronic hepatitis B and C, Helicobacter infection, stone size, and stone number. However, there were significant statistical differences in terms of the mean age and proportion of females between the two groups. The mean age of participants in the CLS group was lower than that of the PGS group (35.7 vs. 54.4, p < 0.01). There was a higher proportion of women in the CLS group than in the PGS group (71.1% vs. 46.4%, p = 0.020).

In the multivariate analysis using the logistic regression model, advanced age had a significant relationship with the PGS group (OR=1.18, 95% CI, 1.07–1.35, p < 0.01), and a borderline significant correlation (OR=0.39, 95% CI, 0.13-1.14, p=0.085) was presented between female sex and the CLS group (Table 3).
Fig. (3). Histogram regarding the distribution of cholesterol component proportion for each classification among the four groups divided by gross findings (A) and the two groups divided based on the gallstone analysis results (B).

Table 1. Results of the composition analysis of the initial four gross morphologic gallstone groups and the final two chemical gallstone groups according to their cholesterol concentration (N=106).

<table>
<thead>
<tr>
<th>Gross Finding Stone Groups</th>
<th>Composition of Stone</th>
<th>Final Classification*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CB (Mean, SD)</td>
<td>CL (Mean, SD)</td>
</tr>
<tr>
<td>Black Pigmented Stone</td>
<td>28 (26.4) 43.6, 32.9</td>
<td>52.7, 39.7</td>
</tr>
<tr>
<td>Brown Pigmented Stone</td>
<td>13 (12.3) 10.0, 5.8</td>
<td>90.0, 5.8</td>
</tr>
<tr>
<td>Mixed Cholesterol Stone</td>
<td>31 (29.2) 24.1, 32.7</td>
<td>78.8, 25.8</td>
</tr>
<tr>
<td>Cholesterol Stone</td>
<td>34 (32.1) 10.5, 7.2</td>
<td>89.5, 7.2</td>
</tr>
<tr>
<td>Total</td>
<td>106 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CB, calcium bilirubinate; CL, cholesterol; CC, calcium carbonate; SD, standard deviation.

*CLS was defined as a stone with a cholesterol component of more than 60%, while a stone with less than 59% cholesterol was defined as PGS.

** Two BPS (Calcium Oxalate crystal 100%) were classified as PGS.

** Two BPS had varying compositions (Calcium carbonate 65%, calcium bilirubinate 20%, mixed bile pigment only 15%), but were classified into PGS due to the absent cholesterol content (0%). ** One BPS (CC 98% + CB 2%) was classified into PGS.

Table 2. Clinical characteristics of the two gallstone groups (CLS vs. PGS) according to composition analysis (N=106).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Data Types</th>
<th>Cholesterol Stone¹ N=76</th>
<th>Pigmented Stone¹ N=30</th>
<th>Total N=106</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean, SD</td>
<td>35.7, 11.5</td>
<td>54.4, 20.8</td>
<td>40.72, 16.71</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Median, min-max</td>
<td>33.0, 15.0-68.0</td>
<td>58.0, 15.0-97.0</td>
<td>37.0, 15.0-97.0</td>
<td></td>
</tr>
<tr>
<td>Sex, F</td>
<td>N, %</td>
<td>54, 71.1</td>
<td>13, 46.4</td>
<td>67, 64.4</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI</td>
<td>Mean, SD</td>
<td>32.3, 8.0</td>
<td>30.7, 13.2</td>
<td>31.9, 9.7</td>
<td>0.439</td>
</tr>
<tr>
<td>Parity</td>
<td>Mean, SD</td>
<td>3.2, 3.1</td>
<td>5.0, 3.7</td>
<td>3.9, 3.2</td>
<td>0.187</td>
</tr>
<tr>
<td>Obesity</td>
<td>Yes, %</td>
<td>38, 50.0</td>
<td>12, 42.9</td>
<td>50, 51.8</td>
<td>0.518</td>
</tr>
<tr>
<td>Education Level</td>
<td>High, %</td>
<td>26, 35.6</td>
<td>8, 28.6</td>
<td>34, 33.7</td>
<td>0.502</td>
</tr>
<tr>
<td>ABO blood type</td>
<td>A, %</td>
<td>16, 22.9</td>
<td>8, 38.1</td>
<td>24, 26.4</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>AB, %</td>
<td>3, 4.3</td>
<td>0, 0</td>
<td>3, 3.3</td>
<td></td>
</tr>
</tbody>
</table>
To establish definite relationships with gallstone development, chemical compositions of each gallstone and that the majority of BRPS and MCS in this study had the same contents as CLS. It has been accepted that there are three different types of gallstones (BLPS, BRPS, and CLS), and they have different pathogeneses [4, 11]. BLPS is caused by abnormal bilirubin metabolism, as seen in cases of chronic hemolytic anaemia, ineffective erythropoiesis, ileal disease, or cirrhosis. Bacterial infection developed in bile duct obstruction can contribute to BRPS formation. CLS can be caused by the disequilibrium of the biliary cholesterol homeostasis, which occurs due to three major pathogeneses, namely hepatic disequilibrium of the biliary cholesterol homeostasis, which can contribute to BRPS formation. CLS can be caused by the disequilibrium of the biliary cholesterol homeostasis, which can contribute to BRPS formation.

### Table 3. Multivariate analysis with a logistic regression model analyzing the risk factors for cholesterol stones and pigmented stones among 106 Gallstone Cases performed Stone Analysis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Data Types</th>
<th>Cholesterol Stone(^1) N=76</th>
<th>Pigmented Stone(^2) N=30</th>
<th>Total N=104</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean, SD</td>
<td>35.7, 11.5</td>
<td>54.4, 20.8</td>
<td>40.72, 16.71</td>
<td>1.18</td>
<td>1.07-1.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sex, F</td>
<td>F:M</td>
<td>2.45</td>
<td>0.84</td>
<td>1.81</td>
<td>0.39</td>
<td>0.13-1.14</td>
<td>0.085</td>
</tr>
<tr>
<td>Obesity, Yes</td>
<td>Yes, %</td>
<td>38, 50.0</td>
<td>12, 42.9</td>
<td>50, 51.8</td>
<td>1.60</td>
<td>0.24-1.45</td>
<td>0.431</td>
</tr>
<tr>
<td>DM, yes</td>
<td>Yes, %</td>
<td>3, 3.9</td>
<td>5, 17.9</td>
<td>8, 7.7</td>
<td>28.00</td>
<td>1.28-1082.92</td>
<td>0.640</td>
</tr>
<tr>
<td>Fatty Liver Disease, yes</td>
<td>Mean, SD</td>
<td>3.2, 3.1</td>
<td>5.0, 3.7</td>
<td>3.9, 3.2</td>
<td>5.42</td>
<td>0.29-0.85</td>
<td>0.716</td>
</tr>
</tbody>
</table>

1. Cholesterol stone (CLS): cholesterol ≥ 60%.
2. Pigmented stone (PGS): cholesterol < 60%.
3. Overweight: ≥ 25 BMI.
4. Pigmented stone: cholesterol < 60%.
5. Abbreviations: OR, odds ratio; CI, confidence interval; SD, standard deviation; M, male; F, female; DM, diabetes mellitus.

### 4. DISCUSSION

This research was a clinical observational study based on medical records in a single institute in UAE that analysed gallstone composition. We could not make any conclusions about the epidemiologic risk factors contributing to the development of gallstones according to their composition, except that advanced age is related to PGS. We realized that gross morphologic findings could not accurately predict the chemical compositions of each gallstone and that the majority of BRPS and MCS in this study had the same contents as CLS but not BLPS.

For the longest time, gallstones have been traditionally classified into three groups (CLS, MCS, and BLPS) [9]. However, many experts have doubted the correlation between these classifications and their pathogenesis, epidemiologic risk factors, and consequently, their usefulness in establishing a prevention plan [10]. It has been accepted that there are three different types of gallstones (BLPS, BRPS, and CLS), and they have different pathogeneses [4, 11]. BLPS is caused by abnormal bilirubin metabolism, as seen in cases of chronic hemolytic anaemia, ineffective erythropoiesis, ileal disease, or cirrhosis. Bacterial infection developed in bile duct obstruction can contribute to BRPS formation. CLS can be caused by the disequilibrium of the biliary cholesterol homeostasis, which occurs due to three major pathogeneses, namely hepatic cholesterol hypersecretion, supersaturation, and gallbladder hypomotility. In addition, numerous other risk factors have been suggested for gallstone development, such as ethnicity [12], genetics [13], age [14], female sex [14], pregnancy or estrogen hormone, obesity [15], metabolic syndromes [16], including insulin resistance [17], rapid weight loss, diet, or parenteral nutrition, low socioeconomic status, alcohol consumption [18], Helicobacter infection [19], vitamin D, and microbiota [20].

Although our study could not reach a reasonable conclusion to prove certain relationships between these variables, except for that between pigment stones and old age, we did generate some useful information that can be used to determine the prevalence and epidemiologic predictors in this region. We found that community-based prevalence and incident data were specific for each ethnic group in this country. Moreover, we determined that the gallstone classification of PGS and CLS should be verified by composition analysis, and we reported the region-specific cutoff values of the CL and CB components for gallstones in UAE nationals. With the above-mentioned information, we can advance our research to find the risk factors for each type of gallstone.

According to our study results, there were more cases of CLS in the hospital-based population than of PGS, and most BRPS can be considered as CLS. We also acknowledge that the gross morphologic classification of gallstones is a very subjective determination. Second, we could not define the risk factors and morbidities in detail because it was a small-sized, retrospective observational study performed in a single center. To establish definite relationships with gallstone development,
we collected comprehensive data regarding comorbidities, such as duration, severity, medication, any treatment of chronic diseases (DM, HTN, hypercholesterolemia, chronic liver disease, renal disease, and vascular occlusive diseases) and the parity history, including detailed gynecologic history, usage of oral contraceptives and/or hormone treatment, and operation history. In order to elucidate the contribution of Helicobacter or other microorganisms, local reports on Helicobacter prevalence, treatment outcomes, resistance rate against clarithromycin, and detailed recent antibiotic treatment history would be necessary. If we could investigate the gene profile of the Helicobacter species from the stomach and gallbladder specimens by real-time reverse transcriptase polymerase reaction, it would make the study more comprehensive.

CONCLUSION
In conclusion, we found 76 CLS (71.7%) and 30 PGS (28.3%) among the 106 stones analysed, based on our classification criteria, which was defined as more and less than 60% of cholesterol content in the stone, respectively. The relationship between old age and PGS development was confirmed in this study.

AUTHORS’ CONTRIBUTION
Byung Hyo Cha, MD is the guarantor of the article.

Specific authors’ contributions:
BH Cha, MJ Park, JY Baeg, SP Lee, and YJ Ahn were responsible for the conception of the study design; BH Cha, DJ Hong, BK Kim, HJ Park interpreted the data; BH Cha, OM Idris, WS Salami collected the data; BH Cha, MJ Park, JY Baeg, SP Lee, and YJ Ahn, DJ Hong, BK Kim, HJ Park, OM Idris, and WS Salami were actively involved in drafting and reviewing the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
The protocol of the present study was approved by the Ministry of Health and Prevention Research Ethics Committee in the UAE (Approval Reference No: MOHAPiDXB-REC/MJO/No.22 2019).

HUMAN AND ANIMAL RIGHTS
No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION
Informed consent was obtained from all the participants included in the study.

STANDARDS OF REPORTING
STROBE guidelines were followed in this study.

AVAILABILITY OF DATA AND MATERIALS
The data supporting the findings of the article is available from corresponding author [B. H. C.] upon reasonable request. The data are not publicly available due to restrictions of their containing information that could compromise the privacy of research participants.

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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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REFERENCES
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