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Original Article

Gallstone Biochemical Analysis: A Key to Unlocking Disease Etiology?

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ABSTRACT

Aim: The widespread use of ultrasound imaging has increased detection rate of gallstones (GSs) in the pediatric age group. However, their etiology remains unclear in some patients. GS analysis of patients who had undergone cholecystectomy in our department were reviewed and the relationships of etiological factors were evaluated.

Materials and Methods: The records of those patients who had undergone cholecystectomy for GS disease in our clinic between November, 2006 and April, 2024 were reviewed retrospectively and demographic characteristics, comorbidities, and stone analysis results were obtained. Statistical analysis was performed using the chi-square test. A p-value <0.05 was considered significant.

Results: Cholecystectomy was performed on a total of 335 patients during the given period. Data for stone analysis were available for 184 patients (105 females, 79 males). The mean age of the patients at the time of surgery was 10.89 (±5.1) years. Stone analysis revealed calcium bilirubinate stones in 104 (56.5%), cholesterol and calcium bilirubinate (mixed) stones in 67 (36.4%), and cholesterol stones in 15 patients (8.1%). A statistically significant difference was found when stone types were analysed by gender (female/male: 105/79; p<0.015). The etiologic factor for GS formation was identified in 56 patients (30.43%); 31 had haemolytic disease and calcium bilirubinate stones were significantly more common in those patients (p=0.006). Additionally, when the weight percentiles for age were evaluated for the 125 patients (67.9%) with available weight data, it was found that cases with cholesterol stones had significantly higher weight percentiles (>90th percentile, p<0.0001).

Conclusion: In our series, cholesterol stones were more common in overweight children, while calcium bilirubinate stones were more common in those with haemolytic diseases. It appears that the composition of the stones can provide clues into understanding the etiology of cholelithiasis.

Keywords: Cholecystectomy, cholelithiasis, gallstone, gallstone analysis, pediatrics

Introduction

Cholelithiasis, once considered uncommon in pediatric populations, has become increasingly recognized due to improved access to imaging modalities such as ultrasonography and growing clinical awareness. Gallstones (GSs) are solid concretions which develop within the biliary system and are primarily composed of cholesterol or bilirubin. Their formation is thought to result from disturbances in the physicochemical balance of bile, often driven by altered cholesterol or bilirubin metabolism (1). The crystals which accumulate and precipitate in the bile as a result of this imbalance are induced by various etiological factors. Therefore, the chemical composition of the GS can indicate the factors involved in their development. In GS formation,

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cholesterol primarily accumulates due to a supersaturation of cholesterol in the bile, while calcium bilirubinate precipitates due to defective bilirubin conjugation, leading to its accumulation (2-4). Additionally, calcium carbonate, calcium phosphate, and infection stones can also be minor components in GS formation. However, GSs are generally categorized into two main types based on their primary components: cholesterol and pigment stones. Although cholesterol is the main chemical compound identified in GS formation, calcium bilirubinate is the primary chemical compound in pigment stone formation (4).

The prevalence and chemical composition of GS vary among different populations, suggesting that multiple etiological factors contribute to their formation. The chemical structure of GS may provide valuable insights into the underlying mechanisms of cholelithiasis. In pediatric patients, the widespread use of imaging techniques such as ultrasonography has led to an increased detection of GS, particularly in those presenting with symptoms such as abdominal pain, postprandial discomfort, or unexplained irritability in infants. Although several risk factors, such as obesity, haemolytic diseases, parenteral nutrition, and certain medications have been associated with GS formation in children, a notable proportion of cases remain idiopathic.

The aim of this study was to investigate the chemical composition of GS in pediatric patients who were operated on at our centre through an in-house laboratory-based analysis, and to explore the possible associations between stone type and clinical variables such as age, sex, body weight, and comorbid conditions. We believe that understanding the detailed composition of GS can enhance our knowledge of pediatric cholelithiasis and contribute to better etiological classification and individualized management strategies.

Materials and Methods

Study Design

The records of those patients who had undergone cholecystectomy for GS in our clinic between 2006 and 2024 were retrospectively reviewed. All patients underwent laparoscopic cholecystectomy. Based on the operating surgeon's preference and patient-specific considerations, either a standard four-port or a three-port laparoscopic technique was utilized. All procedures were performed by experienced pediatric surgeons under general anaesthesia, following standard aseptic protocols.

The demographic characteristics, clinical history, comorbidities, medication use, anthropometric measurements, and stone analysis results of the patients were retrospectively and comprehensively evaluated using data obtained from archived patient files and the electronic medical record system. Those patients for whom stone analysis was performed and recorded were compiled. Patients with choledochal cysts and malignancies of the gallbladder (GB), as well as those whose body weight at the time of operation was not available making percentile calculations impossible were excluded from this study. Additionally, cases with GB polyps or those who underwent cholecystectomy in conjunction with other procedures were also not included. Weight data were obtained for all accessible patients and included in the analysis and all patients received preoperative ursodeoxycholic acid (UDCA) therapy initiated by the pediatric gastroenterology department. UDCA was administered at a standard dosage of 30 mg/kg/day, regardless of the patient's symptomatic status. While treatment dosage was uniform across the cohort, data on the exact duration of UDCA therapy varied between patients and were not consistently documented.

Stone Analysis

One of the most distinctive aspects of this study lies in the real-time biochemical analysis of the GS, performed intraoperatively at our institution. Unlike many previous reports where stone specimens were sent to external laboratories with delayed reporting, our clinical workflow enabled the immediate on-site analysis of the GS composition. Following completion of cholecystectomy, the excised GB was directly delivered to a laboratory located within our surgical unit.

Under the supervision of trained laboratory staff, the stones were promptly examined using the Olympus CX43 stereo microscope system, allowing high-resolution visualization of the surface morphology. As part of the preparation process, the stones were first crushed using a mortar and pestle to reduce their size. This powdered sample was then combined with ammonia, thus facilitating the identification of the calcium bilirubinate and cholesterol components through a chemical reaction. The entire analysis was typically completed within approximately 5 minutes, enabling rapid insight into the biochemical nature of the stones while the surgical team remained present. This integrated, near real-time diagnostic approach not only ensured the reliability of the data but also opened up the possibility of establishing early correlations between GS composition and the underlying disease etiology. The ability to perform stone analysis at the point of care, using both stereomicroscopic and chemical evaluation techniques,

represents a novel and practical model for future studies which aim to link biochemical stone profiles with patient-specific risk factors and metabolic conditions.

Ethical Approval and Helsinki Information

This study was approved by the study was obtained from the Ethical Review Committee of Ege University Faculty of Medicine (approval no.: 2024-2772 24-4.1T/49, date: 25.04.2024). All admissions and surgical procedures were performed after receiving informed consent from the family/parents/caregivers.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 21.0 software for Windows (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp., USA). Statistical analysis was performed using the chi-square test, and a p-value <0.05 was considered statistically significant.

Results

Between November 2006 and April 2024, a total of 335 patients underwent cholecystectomy in our clinic. We confirm that the surgical method applied in all patients was laparoscopic cholecystectomy. Importantly, no intraoperative or postoperative complications were observed in any of the patients, and all surgeries were completed successfully without the need for conversion to open surgery. A total of 184 patients with stone analysis results and weight data available in medical records were included in this study. The demographic data of the patients are summarized in detail in Table I. All patients referred to us from pediatric gastroenterology were initially started on UDCA for litholysis, and this group included asymptomatic patients.

Of our patients, 104 (56.5%) had calcium bilirubinate stones, 67 (36.5%) had both cholesterol and calcium bilirubinate stones, and 13 (7%) had cholesterol stones (Figures 1-3). Four patients had infection stones in addition to calcium bilirubinate stones. A statistically significant difference was observed in the types of stones when analysed by gender (female/male: 105/79; p<0.015). The majority of stones in male patients were calcium bilirubinate stones, while cholesterol stones were significantly more common in female patients.

Among the 56 patients (30.43%) for whom an etiological factor could be identified, 31 had haemolytic disease. The most common haematologic condition was hereditary spherocytosis, followed by thalassemia major, acute myeloid leukaemia, acute lymphoblastic leukaemia, and glucose-6-phosphate dehydrogenase deficiency. All stones in this group were calcium bilirubinate stones. It was found that calcium bilirubinate stones were significantly more common in those patients with haemolytic disease (p=0.006). Additionally, when the weight percentiles for age were evaluated for the 125 patients (67.9%) with available weight data, it was found that cases with cholesterol stones had a statistically significant higher weight percentiles

Table I. Demographic and clinical findings of the patients (n=184)

Parameters	Value
Gender F/M (%)	105 (57.1)/79 (42.9)
Mean age ± SD, years (range)	10.89±5.1, (0.5-19)
Obesity (>90th percentile), n (%)	46 (40.7)
Identifiable etiology n (%) Haematologic n (%) Non-haematologic conditions n (%) (e.g., FMF, portal hypertension, PCOS, and multiple sclerosis...)	 56/184 (30.43) 31 (55.35) 25 (44.65)

F/M: Female/male, FMF: Familial mediterranean fever, PCOS: Polycystic ovary syndrome, SD: Standard deviation

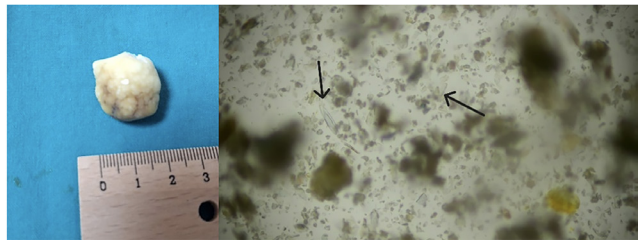

Figure 1. Cholesterol stone, macroscopic and microscopic view- arrows: cholesterol plaques

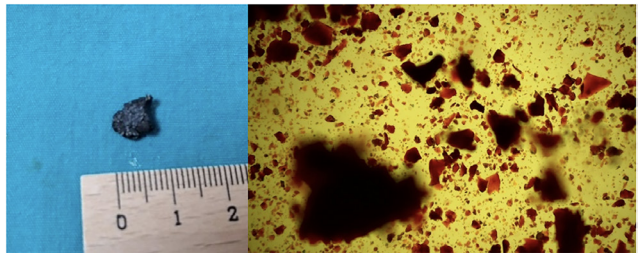

Figure 2. Calcium bilirubinate stone, macroscopic and microscopic view

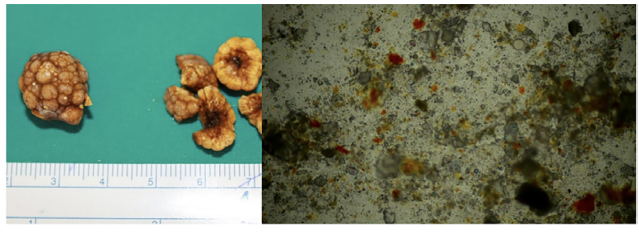

Figure 3. Mixed stones, cholesterol+calcium bilirubinate macroscopic and microscopic view

(>90th percentile, p<0.0001). Also, among the patients with available weight data (n=125), the age-appropriate weight percentile classification revealed that 46.0% (n=52) were above the 97th percentile, 9.7% (n=11) were below the 3rd percentile, and the remaining patients were distributed across intermediate percentile ranges. This distribution indicates a predominance of overweight and obese patients in the cohort. Although body mass index (BMI) could not be calculated due to the lack of height data, weight percentiles for age served as a useful surrogate indicator of nutritional status (Table II).

Discussion

The incidence of GSs detected in children has increased with the widespread use in non-invasive imaging techniques and a global increase in dietary disorders and obesity (5,6). Studies have shown that the etiology of GS formation varies between populations, although the primary factors contributing to these differences remain a subject of debate (7). There is also no clear consensus on whether cholecystectomy should be performed on asymptomatic patients (8).

In our study, the age group most frequently affected by cholelithiasis was 10-16 years, with a predominance of females in this age range. Calcium bilirubinate stones were the most common type of GS observed. Haematologic diseases have been identified as the most frequent risk factor for GS formation, with incidences reported to range from 9% to 50% (9). The higher incidence of cholesterol stones in females was statistically significant, possibly related to a decrease in bile acid reserve and an increased cholesterol saturation of bile in females during puberty when compared to males (10,11).

In pediatric patients, data regarding post-cholecystectomy outcomes remain extremely limited. These findings are primarily based on adult populations, and their applicability to the pediatric age group remains uncertain due to the lack of long-term, large-scale studies in children (12). While some retrospective case series have noted non-specific gastrointestinal complaints such as abdominal pain, diarrhoea, or dyspepsia after surgery, there is currently no conclusive evidence establishing a direct association with long-term complications such as post-cholecystectomy syndrome, colon cancer or persistent symptoms. Future research could focus on investigating the relationship between stone analysis results and long-term postoperative symptoms, as well as gathering data on post-cholecystectomy syndrome in children (13,14).

Therefore, future research should aim to address these knowledge gaps by incorporating prospective, longitudinal follow-up protocols, ideally with standardized symptom questionnaires, imaging modalities, and biochemical monitoring. In addition, evaluating the potential relationship between GS composition and long-term postoperative outcomes may provide valuable insights in this field. Determining whether specific stone types are associated with a higher risk of complications could contribute to the development of future risk stratification models, follow-up strategies, and personalized treatment plans. Therefore, we are considering this topic as one of the focus areas for our future research.

The S3 guidelines recommend UDCA treatment in asymptomatic patients (15). All patients referred from pediatric gastroenterology to our clinic were started on UDCA for litholysis, and no significant difference was observed between the symptomatic and asymptomatic patients. Although Corte et al. (16) reported significant relief in symptomatic cases, they argued that UDCA does not offer a 100% cure for GS treatment. A study by Baran et al. (17) on 74 children supported these findings, suggesting that UDCA treatment could be initiated preoperatively in asymptomatic patients without haematologic diseases (17).

Table II. Comparison of patients according to stone type

Parameters	Calcium stones	Mixed stones	Cholesterol stones
n (%)	104 (56.5)	67 (36.5)	13 (7.0)
Female gender, n (%)	50 (48.1)	43 (64.1)	12 (92.3)
Mean age, mean ± SD, years (range)	8.3±4.4 (0.5-18)	14.1±2.7 (7-19)	14.7±2.04 (11-17)
Obesity, (>90th percentile), n (%)	11 (10.5)	25 (37.3)	10 (76.9)
Haemolytic disease (Hereditary spherocytosis, ALL, AML, thalassemia major, G6PD deficiency), n (%)	31 (29.8)	None	None
UDCA use, n (%)	104 (100)	67 (100)	13 (100)

SD: Standard deviation, ALL: Acute myeloid leukaemia AML: Acute lymphoblastic leukaemia, G6PD: Glucose-6-phosphate dehydrogenase, UDCA: Ursodeoxycholic acid

It is important to note that there is still no clear evidence on whether certain specific characteristics of GS, such as their size, number, or composition, are influenced by the duration of UDCA treatment. While our study included patients who received a standardized dosage, the variation in treatment durations and the limited longitudinal data preclude any definitive conclusions on this matter. Further prospective studies with longer follow-ups, incorporating serial imaging and biochemical monitoring are needed to clarify these potential associations in order to better evaluate the effects of preoperative UDCA, thereby providing stronger evidence for its use in pediatric patients. In conclusion, the treatment of GSs in pediatric patients remains a topic of ongoing debate (17).

Study Limitations

This study has several limitations which should be acknowledged. First, due to its retrospective design and the fact that some patients were referred to our centre after initial diagnosis, complete preoperative clinical and laboratory data were not available for all cases. Specifically, cholesterol and triglyceride levels were not routinely measured across the entire cohort, which limited our ability to comprehensively evaluate certain metabolic risk factors. Additionally, consistent data regarding the duration of UDCA therapy prior to cholecystectomy were not available. Although all patients received UDCA at a standardized dose of 30 mg/kg/day, due to insufficient documentation, the variability in treatment duration could not be assessed. Consequently, it was not possible to determine whether the length of medical therapy had any effect on the GS characteristics, such as their size, number, or their chemical composition. Another important limitation was the lack of height data for most patients, which made it impossible to calculate BMI and Z-scores which precluded obesity classifications based on BMI percentiles. Instead, we used age-appropriate weight percentiles in order to assess nutritional status. While a high BMI is a known risk factor in adult populations, both elevated weight percentiles and haematologic disorders are recognized contributors to cholelithiasis in children (6,18). Finally, cholecystectomy rates increase with age due to risk factors such as haematologic diseases, a family history of GS, cystic fibrosis, cephalosporin treatment, and obesity (16,19). Although we investigated these risk factors, information regarding a family history of cholelithiasis was not available in this study. These limitations highlight the need for future prospective studies with standardized data collection and long-term follow-ups.

Conclusion

Our study was a single-centre study with the longest duration and the highest number of patients regarding the value of stone analysis on determining etiology which had been conducted to date. Consequently, in our series, it was shown that cholesterol stones were more common in overweight children and calcium bilirubinate stones were more common in haemolytic diseases. These findings were found to align with the literature, and it was observed that the content of the stones could be an important clue into understanding the etiology of cholelithiasis.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Ethical Review Committee of Ege University Faculty of Medicine (approval no.: 2024-2772 24-4.1T/49, date: 25.04.2024).

Informed Consent: All admissions, surgical procedures were performed after informed consent of the family/parents/caregivers.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.C., Ü.Ç., A.Ç., M.O.E., Concept: M.C., A.Ç., M.O.E., Design: M.C., M.O.E., Data Collection or Processing: M.C., Ü.Ç., T.K., A.Ç., M.O.E., Analysis or Interpretation: M.C., T.K., M.O.E., Literature Search: M.C., Writing: M.O.E.

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