Original Article

The Chemistry of Gallstones: Linking Chemical Composition to Gross Morphology and Treatment Modality in a Southern Iranian Population

Neda Soleimani[1*], Farideh Fesahat[2], Sahand Mohammadzadeh[2], Kimiya Kazemi[3], Davoud Soleimani[4], Keyhan Minaee[5], Ali Ahmadimooseloo[6]

[1] Assistant Professor of Pathology, Fellowship of Gastrointestinal Pathology, Department of Pathology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

[2] Resident of Pathology,Department of Pathology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

[3] Bachelor of Genetics, Department of Pathology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

[4] MS in Food Safety and Quality Control, Center of Policy Planning, Shiraz University of Medical Sciences, Shiraz, Iran

[5] Bachelor in Medical Immunology, Department of Pathology, Shiraz Transplant Center, Abu Ali Sina Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

[6] Bachelor in Medical Laboratory, Department of Pathology, Shiraz Transplant Center, Abu Ali Sina Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Background:
The management of gallstone disease necessitates a thorough understanding of the various stone types and their chemical composition. While studies on gallstones have been conducted worldwide, no research has been performed in southern Iran.

Materials and Methods:
This cross-sectional study analyzed 164 cholecystectomy specimens from patients with symptomatic gallstones over a 6-month period. Demographic data, stone type, histological diagnosis, and chemical composition of blood and stones—including cholesterol, calcium, phosphate, and bilirubin—were examined using an autoanalyzer and manual methods.

Results:
Most patients (78.7%) were female, 21.3% were male, and the average age was above 40 years. Mixed stones were the most common type (52.4%), followed by cholesterol and pigment stones. All stone types were more prevalent in women. Patients over 40 were more likely to have mixed and pigment stones, whereas younger patients had a higher incidence of cholesterol stones. Chronic cholecystitis was the most frequent histological finding (86%), followed by acute cholecystitis. One case demonstrated carcinoma in situ. Cholesterol concentration was highest in cholesterol stones ($P<0.0001$), whereas calcium and bilirubin levels were highest in pigment stones ($P<0.0001$). Only bilirubin levels showed a significant correlation with stone type in blood analysis.

Conclusion:
Different geographical regions, as well as various areas within a country, experience distinct types of gallstones. Accurate classification of gallstones is essential for optimal gallstone treatment, highlighting the importance of designing such research in every region.

Keywords: Chemical analysis, Gallbladder, Gallbladder disease, Gallstones

please cite this paper as:
Soleimani N, Fesahat F, Mohammadzadeh S, Kazemi K, Soleimani D, Minaee K, Ahmadimooseloo A. The Chemistry of Gallstones: Linking Chemical Composition to Gross Morphology and Treatment Modality in a Southern Iranian Population. *Govaresh*.2025;30:62-67.

**Main Corresponding Author:*
Neda Soleimani, MD
Department of Pathology, Shiraz Transplant Center,
Abu Ali Sina Hospital, Shiraz University of Medical
Sciences, Shiraz, Iran
Telefax : + 98 7132301784
Email: Soleimani_n@sums.ac.ir

Received: 12 Feb. 2025
Revised: 11 Jun. 2025
Accepted: 12 Jun. 2025

INTRODUCTION

Gallstone disease, or cholelithiasis, is the formation of crystalline deposits in the gallbladder or bile ducts. It remains one of the most common biliary disorders, with a prevalence of 10-15%. Although its mortality rate is low, it has significant economic and health implications due to high morbidity. The most common symptom is colicky epigastric and right upper quadrant abdominal pain. However, the gallstones can lead to complications such as choledocholithiasis, gallstone ileus, and acute gallstone pancreatitis. Risk factors include age and female sex, obesity, dyslipidemia, diet, ethnicity, diabetes mellitus, pregnancy, and hemolytic diseases (1-7).

Gallstone formation is a multifactorial process. Total cholesterol is the major component, and the minor components include bilirubin, phospholipids, triglycerides, bile acids, fatty acids, calcium, magnesium, and various trace elements. Key factors contributing to stone formation include bile supersaturation, bile concentration inside the gallbladder, crystal nucleation, and abnormal gallbladder motility. Among these, bile cholesterol supersaturation is the most important factor (8, 9).

Gallstones are classified into three main categories based on gross features: cholesterol, pigment, and mixed stones (8). Pure cholesterol stones are typically solitary, round, bluish-white, and composed of cholesterol crystals. Pigment stones are multiple, small, and dark brown to black, composed of bilirubin aggregates, with signs of bacterial infection in some of them. Mixed stones are small, numerous, round, or faceted and vary in composition (8,10-12).

Ultrasonography is the preferred diagnostic procedure due to its high sensitivity, ranging from 90% to 95%. Other diagnostic approaches include computed tomography (CT) scans and magnetic resonance cholangiopancreatography (MRCP). These approaches are ineffective for determining stone composition (5,10).

Understanding the chemical composition of gallstones is crucial for both prevention and medical treatment, as it determines the effectiveness of therapeutic options such as oral dissolution therapy versus cholecystectomy.
The purpose of this study was to investigate the chemical composition of different types of gallstones. We also looked at the relationship between the chemical composition of the stones and demographic data, gross morphology, histological findings from cholecystectomy specimens, and blood analytes.

MATERIALS AND METHODS

This cross-sectional prospective study was conducted at Abu-Ali Sina Hospital (Shiraz, Iran), a specialized gastrointestinal and transplantation center, from April 2022 to November 2022. The study was designed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1400.308). All cholecystectomy specimens containing gallstones were included, while those without stones or without concomitant blood samples were excluded.

Patients' demographic data, including sex and age, were retrieved from their medical records. The stones were classified grossly using the Japanese classification method as cholesterol stones (pale yellow/whitish), pigment stones (black/brownish-black), and mixed stones (brownish-yellow or greenish with laminated layers) (13).
The gallstones were washed, air-dried, and powdered for chemical analysis. Different solvents were used based on the ionic constituents being analyzed. To determine total cholesterol and bilirubin, 30 mg of the dried gallstone powders were taken in a test tube and dissolved in 3 mL of an acidified methanol-chloroform mixture (1:1 v/v) containing 1% of 1N HCl. The tubes were kept in a boiling water bath for two minutes. To determine calcium and inorganic phosphate, 30 mg of the gallstone powder was dissolved in 3 mL of 1 N HCl in a graduated 10 mL tube, and its final volume was made up to 10 mL with distilled water. The tubes were kept in a boiling water bath for 1 hour (4). The obtained stone solutions were used to determine chemical analytes. Chemical analysis was done manually with a spectrophotometer according to the kit's instructions for calcium, inorganic phosphate, total bilirubin, and cholesterol. Simultaneously, serum specimens of the patients were analyzed for the same analytes automatically, using the DIRUI 1200 autoanalyzer and the same commercial kits. Table 1 presents the details of the commercial kits used. Furthermore, the hematoxylin and eosin (H&E) slides of the gallbladders were assessed, and the histological diagnoses were determined (11).

Statistical analysis

Data were analyzed using IBM SPSS (version 25.0). Quantitative variables were expressed as mean ± SD, frequency, and percentage. Statistical significance was set at a P value < 0.05. The Kruskal-Wallis test was used for comparisons among stone types, while Chi-square and Fisher's exact tests were used for categorical data.

Table 1. Characteristics of methods used in the study

Analyte	Method	Reagent	Wavelength (nm)	Analytical Sensitivity (mg/dL)	Linearity Limit (mg/dL)
Calcium	CPC	BIOREX	500	0.2	20
Inorganic Phosphate	Molybdalate	BIOREX	340	0.2	20
Bilirubin	Jendrassik- Grof	BIOREX	546	0.1	25
Cholesterol	CHOD/PAP	BIOREX	546	5	500

CPC: cresolphthalein complexone; CHOD-PAP: cholesterol oxidase/peroxidase.

RESULTS

Gallstones were collected from 164 patients who underwent cholecystectomy. Women comprised 79% of cases. Most gallstones were mixed stones (52%), followed by cholesterol stones (29%) and pigment stones (19%). Examples of each stone type's typical gross morphology are displayed in Figure 1.

All stone types were more prevalent in women. Following mixed stones, patients over 40 were more likely to have pigment stones, whereas younger patients were more likely to have cholesterol stones (Figure 2).

The details of the clinical data and gross features of the specimens are shown in Table 2.

The most common histological finding was chronic cholecystitis (86%), followed by acute cholecystitis (13.4%). In addition, one case (0.6%) showed adenocarcinoma in situ. Figure 3 and Table 3 provide examples of microscopic characteristics, as well as further histological diagnoses.

The calcium, cholesterol, and bilirubin content of the stones and the stone types were statistically significantly correlated (P<0.0001), as seen in Table 4, with pigment stones having the highest levels of calcium and bilirubin, followed by mixed stones. The largest cholesterol concentration was found in cholesterol stones, followed by mixed stones (P<0.0001). There was no statistically significant correlation between phosphorus level and stone type. Patients with pigment stones had higher serum bilirubin levels, whereas serum calcium, cholesterol, and phosphorus levels were not associated with the type of stone.

Figure 1. The gross morphology of gallstones: A: cholesterol stone; B: pigmented stone; C: mixed stone.

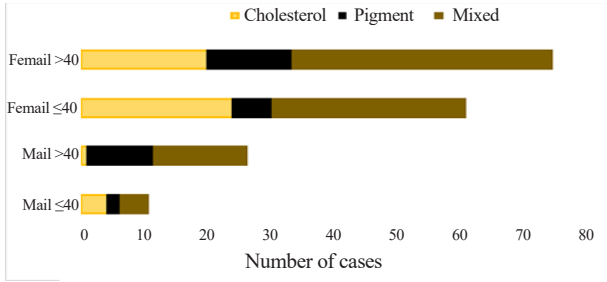

Figure 2. The prevalence of different stones according to sex and age

Table 2. The demographic data and gross morphologic features of the gallstones.

Gender		Age (y/o)		Stone Type		
Female (%)	Male (%)	≤ 40 (%)	> 40 (%)	Cholesterol (%)	Pigment (%)	Mixed (%)
129 (79)	35 (21)	68 (41)	96 (59)	47 (29)	31 (19)	86 (52)

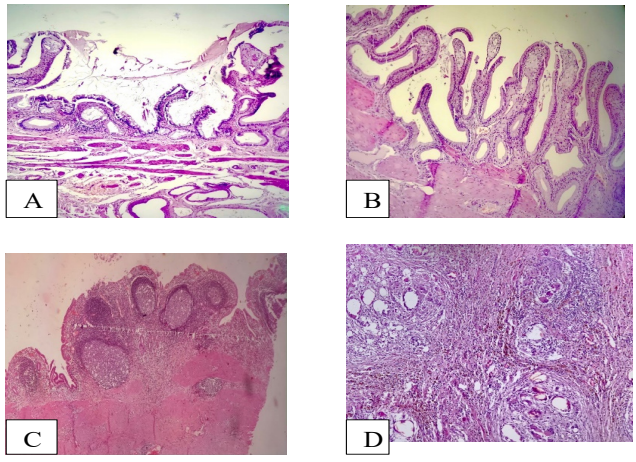

Figure 3. Histopathologic findings A: chronic cholecystitis with intestinal metaplasia (H&E x200); B: Cholesterolosis (H&E x200); C: Follicular cholecystitis (H&E x100); D: Xanthogranulomatous cholecystitis (H&E x200).

Table 3. The histologic findings of gallbladder specimens.

Diagnosis	Pathologic subtypes	Number (%)	Total Number (%)
Chronic Cholecystitis	Follicular cholecystitis	3 (2.1)	141 (86)
	Xanthogranulomatous cholecystitis	4 (2.9)	
	Hyalinizing cholecystitis	1 (0.6)	
	NOS	133 (94.4)	
Acute Cholecystitis	Gangrenous cholecystitis	5 (3)	22 (13.4)
	NOS	17 (10.4)	
Carcinoma	Carcinoma in situ	1(0.6)	1 (0.6)
Additional histological findings	Cholesterolosis	27 (16.4)	NA
	Intestinal metaplasia	7 (4.2)	
	Pyloric metaplasia	1 (0.6)	

NA: not applicable; NOS: not otherwise specified.

Table 4. Chemical analysis of gallstones and serum specimens

Analytes	Stone Type			P-value
	Cholesterol	Mixed	Pigment	
Stone Analysis				
Cholesterol(mg/dL)	694.2±142.9	483.6±66.1	403.8±36.3	< 0.001
Calcium(mg/dL)	6.5±2.7	9.4±3.6	12.2±3.4	< 0.001
Total Bilirubin(mg/dL)	1.6±0.7	2.3±0.8	3.5±0.6	< 0.001
Phosphorus (mg/dL)	2.5±0.5	2.3±0.5	2.5±0.5	> 0.05
Blood Analysis				
Cholesterol(mg/dL)	157.5±42.2	150.8±43.4	136.5±36.2	> 0.05
Calcium(mg/dL)	8.6±0.8	8.7±1	8.8±1	> 0.05
Total Bilirubin(mg/dL)	0.8±0.8	0.7±0.5	1.1±0.8	< 0.001
Indirect Bilirubin(mg/dL)	0.5±0.4	0.5±0.4	0.8±0.7	< 0.001
Direct Bilirubin(mg/dL)	0.3±0.4	0.2±0.2	0.3±0.2	< 0.001
Phosphorus(mg/dL)	3.3±0.6	3.4±0.8	3.4±0.7	> 0.05

DISCUSSION

Gallstone prevalence is increasing, theoretically due to longer life expectancy and dietary changes (14). While gallstone occurrence varies geographically, it remains low in Iran (0.8%-6.3%) compared with Western countries (15,16). This may be attributed to higher fiber intake and lower obesity rates (17).

Our study found a female-to-male ratio of 3.6:1, consistent with data from India, Pakistan, and Jordan (10,18-21). In Western populations, cholesterol stones are more prevalent, whereas pigment stones are more common in Asian and African populations. Additionally, unlike previous studies in other regions of Iran where cholesterol stones were predominant, our findings indicate a higher prevalence of mixed stones. Our findings indicate that dietary patterns and genetic factors may influence gallstone composition in southern Iran (10,18-26). Given the predominance of mixed and cholesterol stones in our region, medical dissolution therapy may be a proper alternative to surgery in select cases.

The progressive trend from cholesterol to pigment stones with aging, as seen in our study, suggests that calcium and bilirubin deposition increase as stones mature. This aligns with theories of gallstone formation but raises questions about possible environmental or metabolic factors in southern Iran.

Regarding cholecystectomy cases, the role of the pathologist is to determine the stage of the inflammatory process,

assign a name to it (such as acute cholecystitis or chronic cholecystitis), establish the presence and composition of gallstones, describe any associated changes (such as cholesterolosis), and search for the presence of incidental carcinoma (10,27). The predominance of chronic over acute cholecystitis was in line with previous studies (6,28-30). Moreover, our investigation found a low incidence of gallbladder malignancy (0.6%).

The strong correlation between stone composition and gross morphology suggests that chemical analysis may not always be required, as visual classification provides reliable results. The findings of this study are particularly applicable for treatment planning. Since cholesterol-containing stones were the most common, medical therapies such as bile acid dissolution therapy may be an alternative for selected patients. Cholecystectomy, however, is still the standard treatment for symptomatic patients (31-38).

The association between gallstone chemical composition and blood analysis showed that bilirubin levels were considerably higher in patients with pigment stones, supporting the effect of hemolysis in their development. However, serum cholesterol concentration did not correlate with stone composition, suggesting that systemic cholesterol levels alone may not be a direct predictor of gallstone type (3,6).

We found no significant correlation between hyperlipidemia and cholesterol stones, contrary to earlier research that linked the two conditions. This could indicate that other metabolic parameters, such as gallbladder motility or bile composition, are more important in this circumstance.

In addition to adopting more modern approaches, such as mass spectrometry, to detect trace elements that may contribute to stone formation, future research should focus on the genetic and metabolic pathways that influence gallstone formation.

CONCLUSION

Gallstone composition varies across geographical regions and even within different areas of the same country. Proper classification of gallstones is essential for optimized cholelithiasis treatment, emphasizing the need for regional studies. Understanding the chemical composition of gallstones can aid in designing preventive and therapeutic strategies, potentially reducing the need for surgical interventions.

DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare related to this work.

FUNDING/SUPPORT:

None.

ACKNOWLEDGMENTS

Not applicable

AUTHORS' CONTRIBUTIONS

SN, FF, and MS developed the study concept and design and wrote the draft manuscript. MK, SD, and FF set up the tests. KM, FF, KK, and AA did the analyses. SN and FF selected the cases and samples. SN, FF, SD, and MS, with the input of all authors, interpreted the data. All authors read and approved the final manuscript.

REFRENCES:

1. Lammert F, Sauerbruch T. Mechansim of diseases: the genetic epidemiology of gallbladder stones. *Nat clin pract gastroenterol Hepatol*. 2005; 2: 423-33.
2. Völzke H, Baumeister SE, Alte D, Hoffmann W, Schwahn C, Simon P, et al. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. *Digestion*. 2005; 71(2): 97-105.
3. Hayat S, Hassan Z, Changazi SH, Zahra A, Noman M, Zain Ul Abdin M, et al. Comparative analysis of serum lipid profiles in patients with and without gallstones: A prospective cross-sectional study. *Ann Med Surg* (Lond). 2019;42:11-3.
4. Nath K, Bhattacharyya K, Das B. Chemical analysis of gallstones and quantitative analysis of its biochemical components viz total cholesterol, total bilirubin, calcium and inorganic phosphate. *JMSCR*. 2018; 6(6):724-9.
5. Schirmer BD, Winters KL, Edlich RF. Cholelithiasis and cholecystitis. *J Long Term Eff Med Implants*. 2005;15(3):329-38.
6. Nagaraj S, Paul P, Kumar M. Risk Factors and the Biochemical Evaluation of Biliary Calculi in Rural Kolar, Karnataka, India: A Rural Perspective of an Urban Disease. *J Clin Dig Res.* 2012, 6(3):364-8.
7. Jaraari A, Jagannadharao P, Patil T, Hai A, Awamy H, El Saeity S, et al. Quantitative analysis of gallstones in Libyan patients. *Libyan J Med.* 2010;5(1):4627.
8. Singh VK, Jaswal BS, Sharma J, Rai PK. Analysis of stones formed in the human gall bladder and kidney using advanced spectroscopic techniques. *Biophys Rev*. 2020;12(3):647-68.
9. Chandran P, Kuchhal NK, Garg P, Pundir CS. An extended chemical analysis of gallstone. *Indian J Clin Biochem*. 2007;22(2):145-50.
10. Sharma R, Soy S, Kumar C, Sachan SG, Sharma SR. Analy-

sis of gallstone composition and structure in Jharkhand region. I*ndian J Gastroenterol*. 2015;34(1):29-37.
11. Goldblum JR, Lamps LW, McKenney JK, Myers JL, Ackerman LV, Rosai J. Rosai and Ackerman's Surgical Pathology. 11th ed. Philadelphia PA: Elsevier; 2018.
12. Siddiqui FG, Memon AA, Abro AH, Sasoli NA, Ahmad L. Routine histopathology of gallbladder after elective cholecystectomy for gallstones: waste of resources or a justified act? *BMC Surg*. 2013;13:26.
13. Kim IS, Myung SJ, Lee SS, Kim MH. Classification and nomenclature of gallstones revisited. *Yonsei Medical Journal*. 2003; 44(4):561.
14. Shareef KM, Omer LA, Garota SA. Predicting the chemical composition of Gallstones by FTIR spectroscopy. *Biomed Pharmacol J*. 2008;1(1).
15. Zamani F, Sohrabi M, Alipour A, Motamed N, Saeedian FS, Pirzad R, et al. Prevalence and risk factors of cholelithiasis in Amol city, northern Iran: A population based study. *Arch Iran Med*. 2014;17(11):750-4.
16. Farzaneh Sheikh Ahmad E, Zavvareh HT, Gharadaghi J, Sheikhvatan M. Prevalence and characteristics of gallstone disease in an Iranian population: a study on cadavers. *Hepatobiliary Pancreat Dis Int*. 2007;6(5):509-12.
17. Massarrat S. Prevalence of gallstone disease in Iran. *J Gastroenterol Hepatol*. 2001;16(5):564-7.
18. Bulo A, Refatllari E, Koci K, Zaganjori R, Isaraj A. Infrared spectroscopy in kidney and biliary stone disease. *Jugoslov Med Biochem*. 2004;23(3):285-8.
19. Jaddou HY, Ammari FF, Shreideh M, Ababneh K. Prevalence and risk factors of gallstone disease among female teachers in Northern Jordan. *Saudi Med J*. 1996;17(2):137-44.
20. Channa NA, Khand FD, Khand TU, Leghari MH, Memon AN. Analysis of human gallstones by flourier transform infrared (FTIR). *Pak J Med Sci*. 2007;23(4):546-50.
21. Jarrar BM, Al-Rowaili MA. Chemical composition of gallstones from Al-jouf province of saudi arabia. *Malays J Med Sci*. 2011;18(2):47-52.
22. Kim E, Lee Y. A descriptive study of gall stone patient‘s dietary habits and nutritional status. *Korean J Comm Nutr*. 2007;12(6):826-77.
23. Pradhan SB, Joshi MR, Vaidya A. Prevalence of different types of gallstone in the patients with cholelithiasis at Kathmandu Medical College, Nepal. *Kathmandu Univ Med J* (KUMJ). 2009;7(27):268-71.
24. Chandran P, Kuchhal NK, Garg P, Pundir CS. An Extended Chemical Analysis of Gallstone. *Indian J Clin Biochem*. 2007, 22(2):145-50.
25. Sekhon M. Morphological spectrum of gallstones disease in 1100 cholecystectomies in North India. *Ind J Surg*. 2005;67(3):140-2.
26. Singh A, Singh G, Kaur K, Goyal G, Saini G, Sharma D. Histopathological changes in gallbladder mucosa associated with cholelithiasis: A prospective study. *Niger J Surg*.2019;25:21-5.
27. Barcia JJ. Histologic analysis of chronic inflammatory patterns in the gallbladder: diagnostic criteria for reporting cholecystitis. *Ann Diagn Pathol.* 2003;7(3):147-53.
28. Ahadi M, Mollasharifi T, Kazeminezhad B, Abdolahi M, Sadeghi A, Khoshnevis J, et al. Histopathologic Features of 1000 Cholecystectomy Specimens. *Int J Cancer Manag*. 2020;13(10):e103522.
29. Holanda AKG, Lima Júnior ZB. Gallbladder histological alterations in patients undergoing cholecystectomy for cholelithiasis. *Rev Col Bras Cir*. 2020;46(6):e20192279.
30. Khan AH, Haroon Ur R, Goraya S. Gallbladder carcinoma; frequency in patients undergoing cholecystectomy for chronic cholecystitis. *Professional Med J*. 2011, 18(1):120-3.
31. Idris SA, Elsiddig KE, Hamza AA, Hafiz MM, Shalayel MHF. Extensive Quantitative Analysis of Gallstones. *IJCM*. 2014;5:42-50.
32. Ti TK, Wong CW, Yuen R, Karunanithy R. The chemical composition of gallstones: its relevance to surgeons in Southeast Asia. *Ann Acad Med Singap*. 1996;25(2):255–8.
33. Nath K, Bhattacharyya K, Das B. Chemical analysis of gallstones and quantitative analysis of its biochemical components viz total cholesterol, total bilirubin, calcium and inorganic phosphate. *JMSCR*. 2018;6(6):724-9.
34. Das B, Khurram Malik A, Rehman A, Samanta S, Ahsan M. Quantitative Analysis of Chemical Composition of Gallstones in North Indian Population (Rohilkhand Region, Uttar Pradesh). *NJIRM*. 2014;5(4):4-12.
35. Al-Sibaai AA, Kensara AM, Baghlaf AO. Element analysis as a strong tool for the determination of gallstones composition. *JKAU*: Sci. 2006;18:33-45.
36. Angwafo III FF, Takongmo S, Griffith D. Determination of chemical composition of gall bladder stones: Basis for treatment strategies in patients from Yaounde, Cameron. *World J Gastroenterol*. 2004;10(2):303-5.
37. Tsai WL, Lai KH, Lin CK, Chan HH, Lo CC, HSU PI, et al. Composition of common bile duct stones in Chinese patients during and after endoscopic sphincterotomy. *World J Gasteroenterol.* 2005;11:4246-9.
38. Schafmayer C, Hartleb J, Tepel J, Albers S, Freitag S, Volzke H, et al. Predictors of gallstone composition in 1025 symptomatic gallstones from Northern Germany. *BMC Gastroenterology*. 2006;6:36.