Original Article

Gallstones Classification by Cross-Sectional Morphology and Using Fourier-Transform Infrared Spectroscopy in Iraqi Kurdish Women

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Abstract

Background: Cholelithiasis is characterized by an abundance of bilirubin or cholesterol in the gallbladder or biliary tract. Gallstones (GSs) made of cholesterol are created when the physico-chemical equilibrium of cholesterol solubility in bile is upset, causing an imbalance in biliary cholesterol homeostasis. It is a surgical problem that general surgeons deal with frequently. Pathogenesis is a painful condition that affects the gallbladder and is associated with an interplay of hereditary and environmental factors. Cholesterol, pigment, or mixed stones are the most common classifications based on their morphology or primary component. **Objectives:** The study's aim is to use Fourier-transform infrared spectroscopy (FTIR) to identify the types of GSs. **Materials and Methods:** Eighty-one GSs surgically removed from the gallbladder were evaluated; FTIR spectroscopy and photographic techniques were used and were classified into three groups based on morphology and different FTIR absorption bands. **Results:** Based on the typical FTIR spectrum features, the constituents discovered include cholesterol, bilirubin, and calcium carbonate, with cholesterol being the most prevalent, followed by bilirubin. The most prevalent stone color was brown, followed by black. Based on FTIR analysis, we divided GSs into three categories: pure cholesterol stones, pure bilirubin stones, and mixed kinds of stones. **Conclusion:** The most prevalent type of GS was the cholesterol stone type.

Keywords: Bilirubin, cholelithiasis, cholesterol, FTIR, mixed stones

Introduction

Gallstones (GSs) are spherical, solid particles made of cholesterol and bilirubin that form in the biliary tract and gallbladder. They vary in size and number; some individuals only generate one or a few huge ones, while others make multiple small ones.[1]

GS disease is a significant global health and surgical problem. Cholelithiasis is a frequent condition characterized by excessive cholesterol or bilirubin in the biliary tract or gallbladder.[2] The alteration in physico-chemical equilibrium of cholesterol's bile solubility leads to an imbalance in biliary cholesterol homeostasis, producing cholesterol GSs.[3] Ultrasound imaging is the most common way to diagnose GS disease.[4] Laparoscopic cholecystectomy is a safe surgical procedure since the majority of complications were mild and well-managed, and a low rate of complications.[5] GSs in the upper right abdomen are frequently painful and associated with symptoms, including stomach discomfort after eating a lot of fatty foods, vomiting, nausea, or abdominal swelling.[6] Gallbladder stones are usually made up of chemical components of bile produced by the liver and secreted in the duodenum throughout digestion.[7] It is getting more prevalent due to the modern lifestyle.[8] According to reports, 13.6% of the population in Iraq has GSs.[9] This is

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significantly greater than the previous estimate of 3.3% in Baghdad.[10] In Kurdistan, there are no available data on GS disease.

In most cases, GS formation is a complex illness involving genetic mutations and environmental interactions. More emphasis should be placed on the major health problems of GSs and the observable increase in the number of cases reported each year.[11] GSs come in a variety of forms. However, they are typically categorized as cholesterol, pigment, or mixed stones (MS). According to their morphology or main component,[12] GSs with cholesterol make up roughly 75% of cases in industrialized nations, while stones with black pigment and brown pigment each make up 20% and 5% of cases, respectively.[13]

Japanese and US researchers have established Fourier-transform infrared spectroscopy (FTIR) as a method of GS analysis, primarily used in extensive studies of GS samples.[14] The surface color, uniformity, and cross-sectional appearance are all evaluated visually. However, classifying GSs solely based on their appearance might lead to issues with subjective analysis and conflicts between morphologic findings and the makeup of the stones in some circumstances.[15]

FTIR is beneficial when examining GS samples from numerous individuals to classify them and investigate the causes of GS formation. Because only a small quantity of compound is required, the method is quick and may be used to identify any form of GS, regardless of crystallinity.[16] Furthermore, due to its remarkable accuracy and cost-effectiveness, FTIR is a beneficial technology in determining the chemical structure of GS; depending on the chemical bonding, the GS molecules produce a characteristic infrared absorption spectrum.[15] Attenuated total reflection (ATR)-FTIR has become a useful technique for researching the composition of biological materials and biomaterial surfaces.[17]

In the Kurdistan region, there is no new or little data about the classification of GSs, apart from the study by Sharee *et al.* in 2008 in Erbil City, which documented the classification of GSs using the FTIR instrument.[18] The primary goal of this study is to use the ATR-FTIR method for the first time to detect the structure of distinct types of GSs in Iraqi Kurdish women.

Materials and Methods

Collection of gallstones

GSs were collected from 81 female patients aged 20–70 years with cholelithiasis undergoing laparoscopic/open cholecystectomy at the Department of Surgery, Garmian Medical City (GMC) hospital in *Kalar*, Sulaymaniyah, Iraqi Kurdistan. Initially, patients had their gallbladders surgically removed; GSs were removed from the gallbladder, placed on clean gauze, and allowed to air dry before being thoroughly cleansed with twice-distilled deionized water. In sterile, dry conditions, all stone samples were kept until analysis.

Visual inspection

Later, a digital image of each GS was obtained after the drying process, and external and internal morphology shots were taken after a scalpel cut the GS in half. To determine whether GSs within the same group (cholesterol, mixed, or pigmented) shared a common morphology, images of GSs from each group were gathered and compared side by side. The visual inspection involved gross observations of the GSs, including their cross-sectional appearance, consistency (hard and soft), and surface texture (smooth and rough). Based on the criteria of the Japanese categorization system, GSs were separated into three groups according to the color and degree of hardness of the stones. Black stones are known as pigment stones, whereas yellow-brown or green stones are known as MS. Stones with yellow and whitish color were classified as cholesterol stones (CS). Most CS have a rough surface, a strong hardness, and a color typically white, yellow, or brownish-yellow. Pigment stones are soft and friable, and have a rough surface, that is, either black or brown. Two different levels can be found in the additional MS subgroup that can be seen in the cross-section. Later, the physical characteristics of each stone group were recorded in weight, numbers, size, shape, color, texture, and surface, and GSs were categorized using morphological criteria after being cross-sectioned using a scalpel. A thorough examination of the exterior surface and the nucleus can separate cholesterol from pigment GSs. Based on their color alone, pigment GSs can be identified as black. GSs with a black color are tiny and amorphous, and when cross-sectioned, they resemble pieces of glass. To determine whether GSs from the same group shared a common morphology, pictures of GSs from each group were gathered and put side by side.

FTIR spectroscopy

According to the FTIR spectrum peak, GS categorization was conducted using the diffuse reflectance spectrum approach. For each measurement, 4–8 mg of powdered stone sample was analyzed using FTIR spectroscopy at frequencies ranging from 400 to 4000 cm^{-1}. Cross-section ATR accessory enables the analysis of neat, control spectra for cholesterol, bilirubin, and calcium carbonate of high quality (99% pure) was obtained from the Biochem Pharma Company (France).

The classification of a stone as mixed is based on the presence of two or more different types of material components and the comparability of their contents. The classification of a stone as simple was given if there was only one major component, such as calcium carbonate and cholesterol. The chemical functional groups of cholesterol and bilirubinate salts were highlighted by their peaks at specific wavelengths by the instrument Jasco FTIR (Shimadzu, United States of America), Model FTIR-6300. In FTIR analysis, when chemical bonds in a sample's polarity interact with electromagnetic radiation in a sample, as happens in infrared spectroscopy, they cause light radiation to interact with the sample.

Statistical analysis

Microsoft Excel was used for data collection and calculation of percentages. Numbers and percentages were used to display the patients' data for category features.

Ethical aspects

The Ethics Committee (No. 6: Jane 20, 2021) of GMC of Education, Department of Biology, oversaw the investigation and adhered to the Declaration of Helsinki.

Results

Cross-sectional morphology is used to categorize GS using digital photography, each form of GS's gall bladder with stone, external, and cross-sectional appearance is shown in Figures 1–3.

A: Pure CS with crystals of cholesterol oriented radially from the center outward. According to the Japanese classification system's criteria, these stones, used to discover the etiopathogenesis of GS disease, were designated as pure cholesterol GS. They appear yellow, white, brownish-yellow, and were round or irregular in shape. They were unique and soft, with smooth and rough surfaces [Figure 2A].

B: Layers of cholesterol and pigment are placed alternately in a crescentic pattern in a mixed CS [Figure 2B]. From the center to the periphery, GS featured crescentic layers of material in shades of dark (black or brown) and light (pale white) were referred to as mixed cholesterol GSs. The stones were green, greenish-brown, and large and irregular in shape. They are mostly hard and multiple with smooth surfaces.

C: Pigment stone: A brittle, black, charcoal gray, or grayish-brown pigment stone with uniformly dispersed pigment particles that had no layers in the profile [Figure 2C]. Pigment stones were hard, layer-free granules that were black, charcoal gray, or grayish-brown, their shape was round or irregular with smooth surfaces and mostly single.

FTIR spectroscopy

Using FTIR spectroscopy to identify the components of gallstones

The systematic classification divided gallbladder stones into three categories based on chemical compositions of different types of GS as revealed by FTIR; these comprised MS, pigment stones, and CS. GS types can be identified by comparing the usual FTIR spectra of samples of GSs to those of three different standards: cholesterol, bilirubin, and calcium carbonate, which are depicted in Figures 4A–C.

The spectrum was analyzed by comparing it to standard control spectra of pure cholesterol, bilirubin, and calcium carbonate standards. The FTIR spectrum in Figure 5A demonstrates that cholesterol crystal

Figure 1: Photographs of gallstones from patients in Kurdistan, Iraq

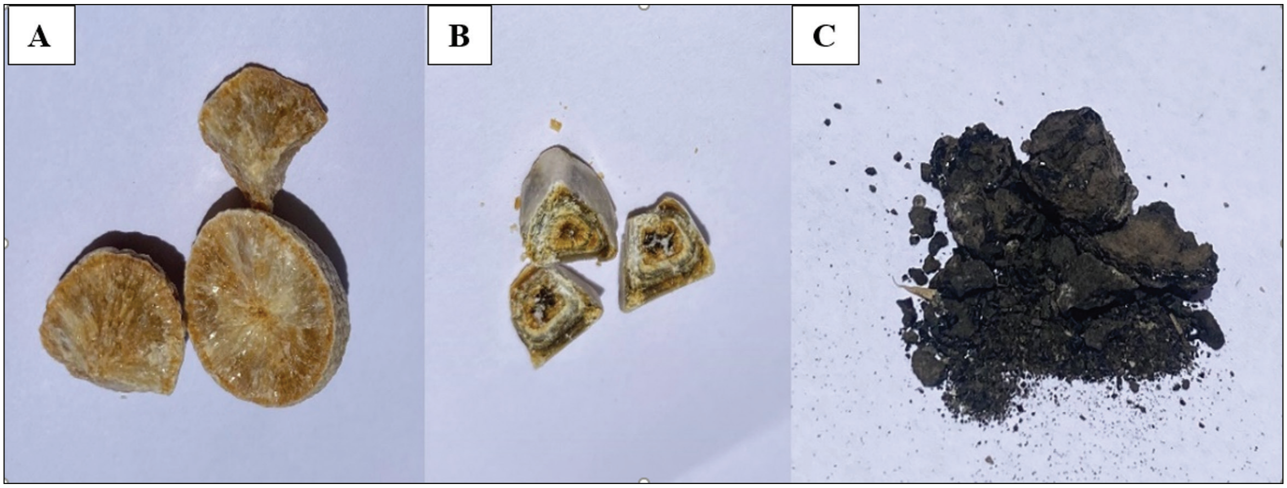

Figure 2: Cross-sectional images of various gallstone types, (A) pure cholesterol, (B) mixed, and (C) pure bilirubin (pigmented)

Figure 3: Photographs of removed gall bladder with stone from patients in Kurdistan, Iraq

Table 1 : IR bands detected in analyzed gallstones

Stone types	Bands							
Cholesterol	3375.43	2931.80	2862.36	1462.40	1049.2			
Mixed	3275.12	2935.65	2862.36	1693.60	1462.40	1363.50	1049.28	680.32
Pigmented	3251.98	1610.67	1364.88	1245.65	781.82	663.51		

dominates the structure of the stones. Cholesterol is distinguished by a broad and powerful band ascribed to O–H stretching at 3375.43 cm^{-1} and a prominent peak at about 2931.80 cm^{-1} due to CH_2 symmetric stretching vibration. The double band (C–C) in the second ring of the cholesterol molecule is identified at 1462.40 cm^{-1} as another peak belonging to the cholesterol molecule. The peak at 1462.40 cm^{-1}, which is linked to the CH_2 and CH_3 bending vibration of the cholesterol molecule, and the peak at 1049.28 cm^{-1}, which is associated with an asymmetric stretching vibration of the CH_2 and CH_3 groups, are used to identify the cholesterol molecule. When two or more different types of material components have a similar composition, the stones are categorized as mixed [Table 1].

Out of 81 GSs, 64.20% were identified as pure CS, 29.63% were MS, and 6.17% were identified as pigment stones. The cholesterol + bilirubin type made up all of the mixed component GSs [Table 2].

The occurrence of various types of GSs in proportion to the age of stone-formers, the high incidence of CS at age (41–50), while MS at age 31–40, and pigmented stones (PS) at age 61–70) [Table 3].

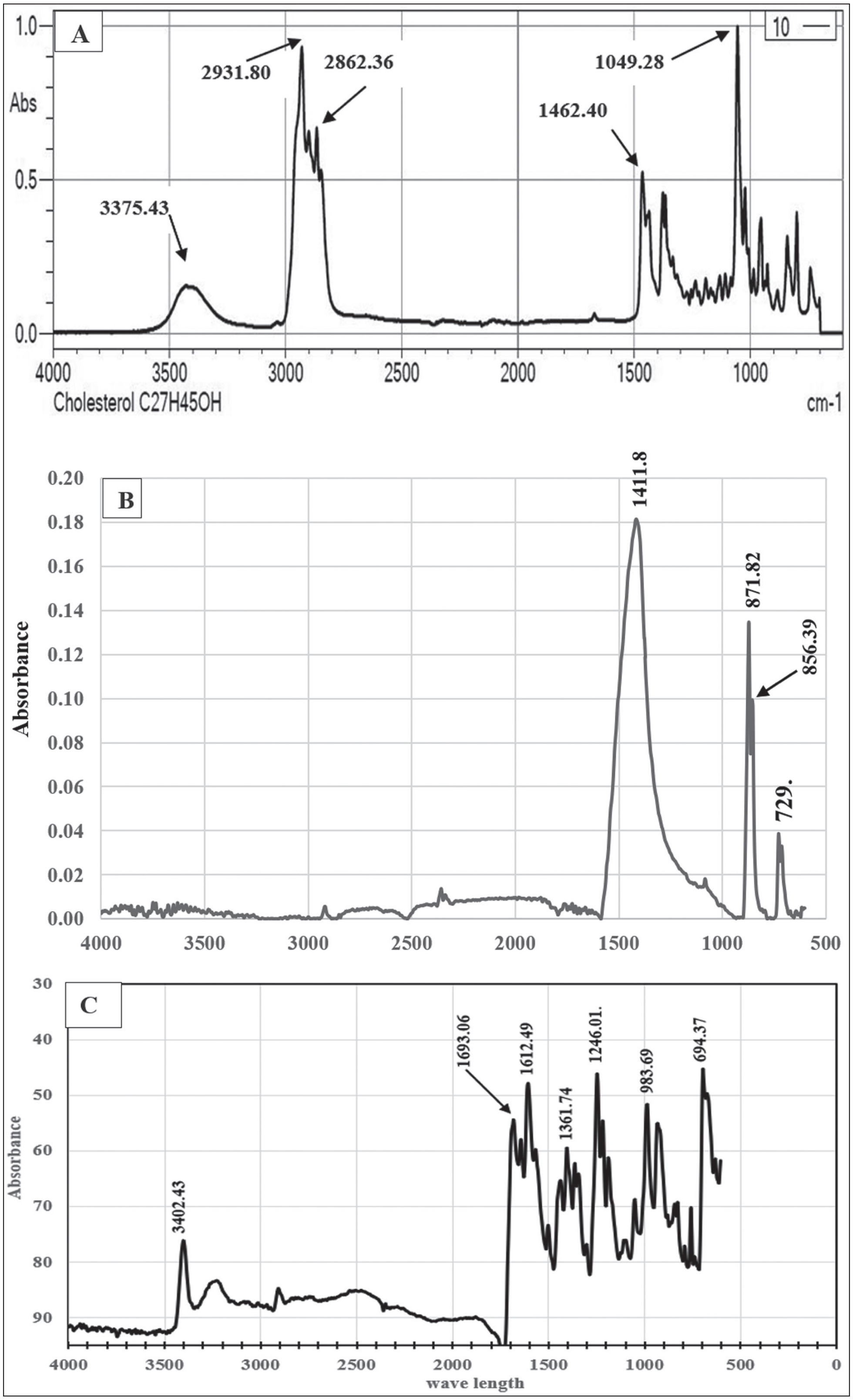

Figure 4: Standard FTIR spectra of pure (A) cholesterol, (B) calcium carbonate, (C) bilirubin

Some metabolic diseases were found in three groups of GSs; the proportion of metabolic diseases was found in the CS group (70%), MS group (23.34%), and PS group (6.66%) [Table 4].

Table 2: Classification of gallstone

Types of stone	Number	%
cholesterol stones	52	64.20
Mixed stones	24	29.63
pigment stones	5	6.17
Total	81	100

The morphology and physical characteristics such as shape (round and irregular), surface (rough and smooth), characters (hard and soft), weight (mean. gm), number (single and multiple), color (yellow, white, brownish-yellow, green, greenish-brown, black) were calculated according to GS classification [Table 5].

Table 3: Distribution of stones according to age

Age group (years)	Cholesterol stones		Mixed stones		pigment stones		Total	
	No	%`	No	%	No	%	No	%
20–30	5	6.17	5	6.17	0	0	10	12.35
31–40	12	14.81	7	8.64	1	1.23	20	24.69
41–50	21	25.93	6	7.41	1	1.23	28	34.57
51–60	7	8.64	2	2.47	1	1.23	10	12.35
61–70	7	8.64	4	4.94	2	2.47	13	16.05
Total	52	64.20	24	29.63	5	6.17	81	100.00

Table 4: Distribution of metabolic disease among gallstone groups

Metabolic disease	CS	%	MS	%	PS	%	Total	%
HTN	9	30	3	10	1	3.33	13	43.33
HT and DM	1	3.33	2	6.67	0	0	3	10.00
DM	5	16.67	2	6.67	0	0	7	23.33
Hypo T	6	20	0	0	1	3.33	7	23.33
Total	21	70	7	23.34	2	6.66	30	100.00

HTN: hypertension, DM: diabetes mellitus, CS: cholesterol stone, MS: mixed stone, PS; pigmented stone

Table 5: Demographic and physical characteristics of different types of gallstone

Morphology	Cholesterol stone		Mixed stone		Pigmented stone		Total
	No	%	No	%	No	%	%
Shape							
Round	32	39.51	8	9.88	3	3.70	53.09
Irregular	20	24.69	16	19.75	2	2.47	46.91
Surface							
Rough	27	33.34	10	12.35	5	6.17	51.86
Smooth	25	30.86	14	17.28	0	0	48.14
Characters							
Hard	31	38.27	13	16.05	5	6.17	60.49
Soft	21	25.93	11	13.58	0	0	39.51
Weight (mean, g)							
Means (g)	2.55	3.48		0.66			
Number							
Single	23	28.40	5	6.17	4	4.94	39.51
Multiple	29	35.80	19	23.46	1	1.23	60.49
Color							
Yellow	15	18.52	–	–	–	–	18.52
White	27	33.33	–	–	–	–	33.33
Brownish-yellow	10	12.35	–	–	–	–	12.35
Green	–	–	14	17.28	–	–	17.28
Greenish-brown	–	–	10	12.35			12.35
Black	–	–	–	–	5	6.17	6.17

Discussion

Different types of GSs contain components, including cholesterol, bilirubin, and other substances. The only effective treatment for GS disease is a cholecystectomy, which involves removing the gallbladder surgically from its location.[19]

The pathogenesis of GS is multifaceted, and determining their chemical content is crucial to understanding their development. This is, as far as we are aware, the initial effort to determine the etiopathogenesis of GS in Kurdish women in Iraq.

The present study systematically analyzed the morphological and chemical components of CS, MS, and PS using photographic and spectroscopic methods. They were classified into many types in the medical literature but mainly classified into three categories based on their cholesterol content: cholesterol GSs, which represent the higher ratio of all GSs with a yellow color and are mostly composed of cholesterol; mixed composition stones, representing the second level of GS types; and pigmented GSs representing the lowest ratio of all GSs and are usually black or brown in color.

The patients were between the ages of 20 and 70. The majority of the patients (24.24%, 81 cases) had an average age of 45.2 years and were in the 40–49 age range. GSs were present in every age group. The majority of patients were female, which is a common occurrence according to another study.[20]

According to the current analysis, CS are more common in Kurdistan than MS or PS. Our findings are well in line with previous research.[18]

Longer life expectancy and different food habits may be contributing factors to the high prevalence of cholesterol GSs in this area. According to literature, females have a high incidence of cholelithiasis.[20] Some studies concluded that MS were the most frequently found stones and the second most prevalent type of stones was CS. These results are inconsistent with the present study.[21]

There is no clear justification for this difference. The complex process of precipitating bile-derived substances such as cholesterol, calcium bilirubinate, and calcium salts of phosphate, carbonate, and palmitate is thought to be involved in the production of GS.[22] In addition to making GS analysis quicker and more affordable, the results were also more thorough than the chemical procedure that had previously been utilized. Cholesterol was the main component of the CS, which was by far the most prevalent stone in the participants of the study. Previous research in the area also claims that cholesterol is the most prevalent component of stone.[18] They noted that pure CS were far more prevalent than cholesterol that had been combined with other substances. In contrast, most of the stones in our study were predominantly composed of cholesterol combined with other components.[23] Taher conducted a study on the GS composition in Baghdad's Medical City Hospital and found that cholesterol constituted up to 50% of the stones.[24] These results are consistent with those of our investigation, which identified cholesterol as the primary component.

According to this study, the prevalence of cholesterol GSs is quickly rising in relation to the worldwide epidemic of insulin resistance, type 2 diabetes, obesity, and metabolic syndrome. A similar study concludes the results.[25]

The current findings of this study, which were consistent with those of earlier research, showed that those who have CS were primarily females between the ages of 20 and 70 and had higher Body Mass Indexes (BMIs).[14] The GSs structure is critical for understanding the etiology of the disease. The mechanism of CS production includes the following steps: Cholesterol supersaturation process occurs first; incorrect bile acid production from the conversion of cholesterol or a disruption in the flow of enterohepatic bile acid are all problems that cause the development of cholesterol GSs.[26]

The mechanism of PS formation occurs when red blood cells are destroyed and bilirubin is released.[27] The color of the stone is related to the color of calcium bilirubinate, which is produced by the reaction between bilirubin and calcium; this process occurs by bacterial-glucuronidase hydrolyzes conjugated bilirubin into free bilirubin.[28]

There are many risk factors that participate in GS formation, such as age, gender, parity, genetics, obesity, body fat distribution, rapid weight loss, type of diet, physical activity, specific medications, and a history of diabetes are all variables that can influence GS formation.[26]

A GS library was created and validated in the current work for the classification of GS materials by FTIR using the zinc selenide ATR accessory. GSs can be quickly analyzed with FTIR.[29] Without any processing or exposure to hazardous substances, the ATR attachment allows for the study of neat stone material. This reduces the amount of time required for the analysis. When compared to the conventional semiquantitative titrimetric–colorimetric approach, the results produced utilizing FTIR technology are more thorough and accurate.[30]

Based on the FTIR absorption properties, we examined the FTIR spectra and photographic images of 81 GSs and divided them into three types. Studies in the past have shown that FTIR analysis offers crucial details on GS contents and may be a useful device for studying the GS formation process. However, due to the modest sample size limit (1, 2 mg).

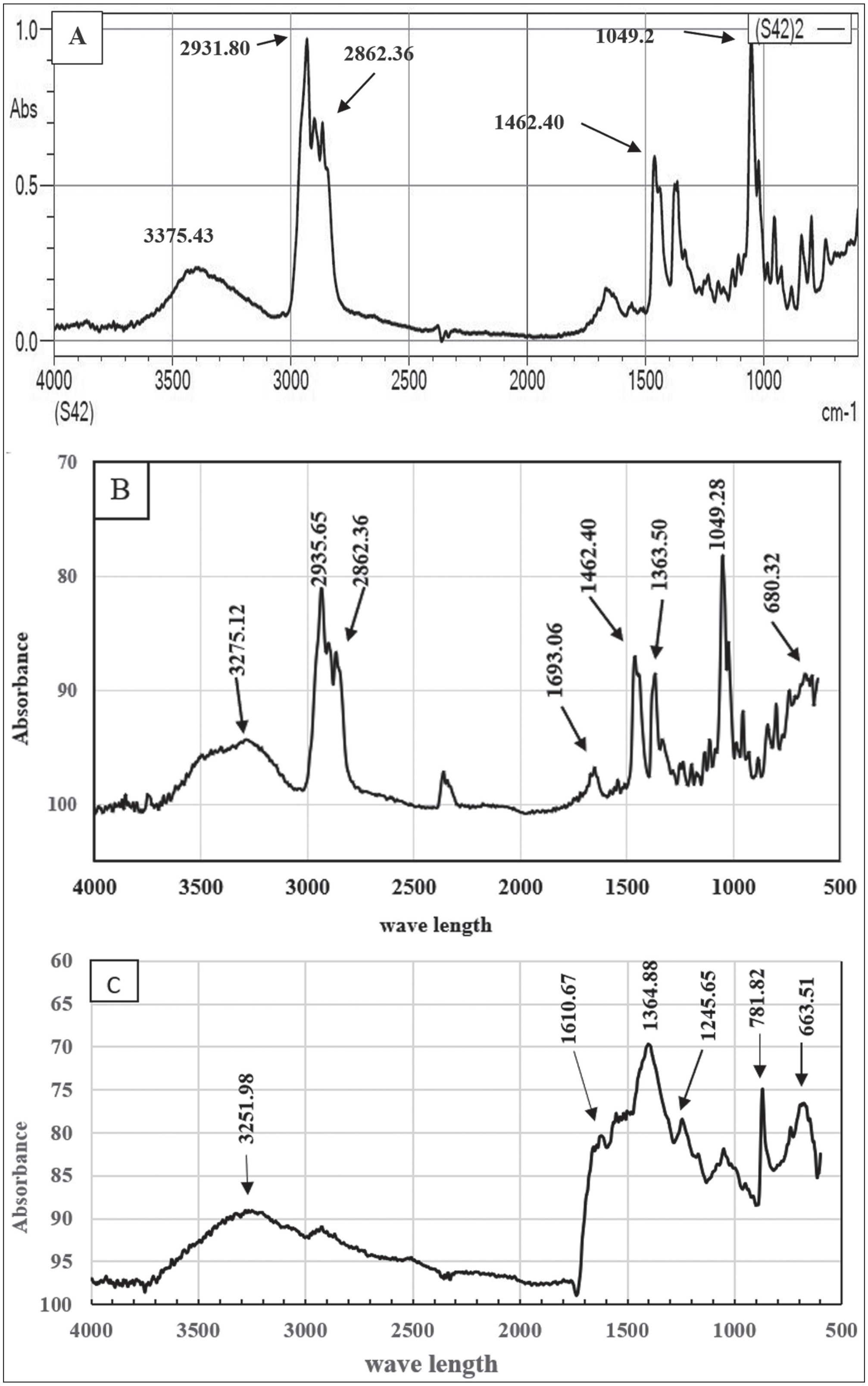

Figure 5: Gallstone spectra of (A) cholesterol, (B) mixed, (C) pigmented

Pure calcium carbonate stones were not found in this study, calcium carbonate stones were a rare sort of stone, mostly affecting children, according to prior research.[18]

The low percentage of pigment stones in this study was parallel to the research by Ravichandran *et al.* who showed a percentage of PS was the least common type.[31] In Iraq, and many other developing countries, parasitic infestation with *Ascaris lumbricoides* is one cause of pigment GSs.[32]

The characteristics of GS showed that 60.49% of patients had hard calculus while soft calculus was seen in 39.51% of cases; a similar study mentioned the same characteristics.[33]

In our study, multiple gallstones were found more frequently (60.49%) than single stones (39.51%), and other studies also confirmed these findings. Single stones were observed in 32/81 (39.51%), and multiple stones were observed in 49/81 (60.49%) patients, as mentioned in other reports.[34]

According to the GSs' color, most of the stones were white (33.33%), followed by yellow (18.52%), green (17.28%), greenish-brown (12.35%), brownish-yellow (12.35%), and black (6.17%), the stones' mean weight was 2.08. ± 4.9 gm. A similar study confirmed these results.[35]

Limitations

Data was only gathered from one hospital, among other limitations. Additionally, the FTIR approach, which is time-consuming, can be used to identify any substances, including trace elements that are found in GSs.

Conclusion

We analyzed 81 GSs by FTIR technique. The FTIR investigations indicated that the cholesterol type of stones occurs more commonly than MS and PS. Age has an influence on the occurrence of GS disease, and the incidences of stone are more common at age 41–50 years. Based on the morphology of GS, it has been found that multiple GSs are more common than single stones.

Recommendations

Further study is needed, and another method is preferred for the classification of gallstone, and a large sample is needed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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