Al-Rafidain J Med Sci. 2026;10(2):127-134.
DOI: https://doi.org/10.54133/ajms.v10i2.2866

AJMS

Online ISSN (3219-2789)

Research Article

Molecular Insights into Gallstone Disease: Association of *FXR* and *CYP7A1* Expression with Gallstone Composition in Iraqi Females

Hala Mohammed Hussien Albakri*, Nuha Joseph Kandala
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq
Received: 23 February 2026; Revised: 11 April 2026; Accepted: 16 April 2026

Abstract

Background: Gallstone illness is a common problem of the biliary system, and its development is known to involve more than one factor. Changes in metabolism, bile composition, and genetic background may all contribute to the formation of gallstones. ***Objective***: The recent work aimed to assess the expression of the bile acid–related genes *FXR* and *CYP7A1* in Iraqi individuals with gallstone illness and to examine their relation to gallstone composition and clinical findings. ***Methods***: A case–control study was carried out on female individuals with gallstone illness and healthy controls. Gene expression of *FXR* and *CYP7A1* was measured using quantitative real-time PCR. Gallstones were examined based on their physical appearance and analyzed via Fourier transform infrared (FTIR) spectroscopy. Clinical and hematological data were collected and statistically analyzed. ***Results***: Individuals showed differences in body mass index and some hematological markers when relative to controls, and a positive family history was more commonly observed. Individuals exhibited reduced expression levels of both *FXR* and *CYP7A1*, particularly among individuals with a familial predisposition to gallstones. Gallstone analysis revealed pigment, cholesterol, and mixed types, with mixed stones being the most common form. ***Conclusions***: The results suggested that gallstone illness contributes to alterations in the expression of bile acid-related genes and heterogeneous stone composition. The prevalence of mixed stones seems to indicate that gallstone development follows multiple mechanisms rather than a singular pathway.

Keywords: *CYP7A1* gene; Gallstone; Gene expression; FTIR; *FXR* gene.

رؤى جزيئية في مرض حصى المرارة: ارتباط مستوى التعبير الجيني لكلٍ من *FXR* و*CYP7A1* بتركيب حصى المرارة لدى النساء العراقيات

الخلاصة

الخلفية: يعد مرض حصى المرارة من المشاكل الشائعة في الجهاز الصفراوي، ويعرف أن تطوره متضمن تداخل عدة عوامل. إذ يمكن أن تسهم التغيرات في الأيض، وتركيب العصارة الصفراوية، والخلفية الوراثية جميعها في تكون الحصوات المرارية. **الهدف**: تقييم مستوى التعبير الجيني لكل من الجينين المرتبطين باستقلاب الأحماض الصفراوية *FXR,CYP7A1* لدى أفراد عراقيين مصابين بمرض حصى المرارة، وتقصي العلاقة بين أنماط التعبير الجيني وتركيب الحصوات المرارية والنتائج السريرية. **الطرائق**: أُجريت دراسة شملت إناثًا مصابات بمرض حصى المرارة إلى جانب مجموعة من الأصحاء كمجموعة ضابطة. تم قياس مستوى التعبير الجيني لكل من *FXR,CYP7A1* باستخدام تقنية تفاعل البوليميراز المتسلسل الكمي بالزمن الحقيقي (qRT-PCR). جرى فحص الحصوات المرارية اعتمادًا على مظهرها العياني، ثم تحليلها باستخدام تقنية مطيافية الأشعة تحت الحمراء (FTIR). جُمعت البيانات السريرية والدموية وخضعت للتحليل الإحصائي. **النتائج**: أظهرت المريضات فروقًا في مؤشر كتلة الجسم وبعض المؤشرات الدموية مقارنةً بالمجموعة الضابطة، كما لوحظت نسبة أعلى لوجود تاريخ عائلي إيجابي للمرض بين المصابات. كذلك تبين وجود انخفاض في مستويات التعبير الجيني لكل من FXR وCYP7A1 ولاسيما لدى ذوات الاستعداد العائلي للإصابة بحصى المرارة. وكشف تحليل الحصوات عن وجود ثلاثة أنماط رئيسية هي الحصوات الصبغية، والكوليسترولية، والمختلطة، وكانت الحصوات المختلطة هي الأكثر شيوعًا. **الاستنتاجات**: أن مرض حصى المرارة يرتبط بحدوث تغيرات في مستوى التعبير الجيني للجينات المنظمة للأحماض الصفراوية، إلى جانب تنوع في تركيب الحصوات. كما أن شيوع الحصوات المختلطة يُعزز الفرضية القائلة بأن نشوء حصى المرارة يتم عبر آليات متعددة متداخلة، وليس من خلال مسار مرضي واحد.

* ***Corresponding author***: Hala M. H. Albakri. Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq; Email: hala.Mohammed1806b@sc.uobaghdad.edu.iq
Article citation: Albakri HMH, Kandala NJ. Molecular Insights into Gallstone Disease: Association of *FXR* and *CYP7A1* Expression with Gallstone Composition in Iraqi Females. *Al-Rafidain J Med Sci.* 2026;10(2):127-134. doi: https://doi.org/10.54133/ajms.v10i2.2866

INTRODUCTION

Gallstone illness is one of the very common health disorders in the human digestive system, and the formation of solid particles, or gallstones, is seen to affect 10 to 20% of the adult population worldwide [1]. This particular condition is defined via the formation of gallstones within the biliary ducts or gallbladder, predominantly composed of cholesterol. Although most individuals with gallstones remain asymptomatic in the early stages of the condition, over 20% will exhibit symptoms such as diarrhea, gastrointestinal pain, vomiting, loss of appetite, fever, nausea, jaundice, or epigastric pain [2]. In the country of Iraq, several epidemiological studies have identified a high incidence of this health disorder, and a study carried out in the Basrah region of the country identified that approximately 13.6% of asymptomatic individuals were identified as suffering from this health disorder through ultrasonography [3]. In addition, a previous study carried

out in the capital city of Baghdad during the years 2004-2005 identified a prevalence of 3.3% in the adult population of the country [4]. These data underscore that gallstone illness is both a global concern and an important health issue within the Iraqi population. Previous studies have observed numerous risk factors for gallstone formation, including age, genetics, gender, ethnicity, pregnancy, diabetes, obesity, and raised total cholesterol levels. [5]. Because of the high levels of high-fat, high-salt, and high-cholesterol foods, as well as the worldwide epidemic of obesity, these factors have been highly correlated with the occurrence of gallstone illness recently [6]. Obesity-related metabolic changes, such as increased cholesterol production and modified bile composition, heighten this risk [7]. As a result, dysregulated lipid metabolism, excessive visceral adiposity, and insulin resistance are considered key pathophysiological factors in gallstone development [8]. These associations of gallstones, both clinically and demographically, have together pointed towards the fact that the formation of gallstones is a multifactorial relationship. Gallstones are generally classified according to their chemical composition. Thus, there are three types of gallstones, namely cholesterol stones, pigment stones, and mixed stones [9]. Among these types of gallstones, cholesterol gallstones are the most common , and they are composed of cholesterol. In contrast, pigment gallstones are dark brown or black in color and are composed of bilirubin calcium compounds, such as calcium bilirubinate. Finally, mixed gallstones are a combination of both cholesterol and pigment stones and are composed of a combination of cholesterol and bilirubinate salts [10]. In addition, gallstones are also classified according to their shape; cholesterol stones are large and round, while pigment stones are small, multiple, and dark in color [11]. Fourier Transform Infrared (FTIR) spectroscopy is often applied for the identification, as well as the characterization, of gallstones. This method allows for the easy and accurate analysis of the various kinds of gallstones, irrespective of the texture and structural differences that they may have. The advantages of the technique include the fact that it is quickly performed, it is inexpensive to use, it has minimal sample requirements, and it is non-destructive, thereby retaining the integrity of the sample. Generally, the range for the infrared spectra is between 4000 and 400 cm^{-1}, in which the absorption peaks correspond to the vibration of certain molecules, enabling the identification of the cholesterol, calcium phosphate, calcium carbonate, and calcium bilirubinate groups [12] and presenting different absorption peaks that can be assigned to specific vibration bands corresponding to the functional chemical groups of cholesterol, calcium phosphate, calcium carbonate, or calcium bilirubinate when compared to IR spectra databases [13]. At the genetic level, nuclear receptors along with essential enzymes control the balance of bile acid and cholesterol in the body; among these is the farnesoid X receptor (*FXR, NR1H4*), which is a nuclear receptor that is activated via bile acids and found in the liver and intestines. It controls the production and transit of bile acids [14], while cholesterol 7α-hydroxylase (*CYP7A1*) is an enzyme that promotes the first and rate-limiting reaction in the classical bile acid synthesis pathway, where it converts cholesterol into bile acids [15]. Therefore, the activation of *FXR* results in the inhibition of *CYP7A1* transactivation [16]. Dysregulation of *FXR* signaling or *CYP7A1* activity impairs bile acid pool size and cholesterol solubility, resulting in cholesterol crystallization and gallstone formation. *FXR* and *CYP7A1* collaborate together to create a regulatory axis that is crucial for the formation of gallstones. However, few studies have aimed to combine molecular markers such as *FXR* and *CYP7A1* with FTIR-based compositional data. Filling this gap may offer new perspectives on the metabolic and genetic connections that contribute to gallstone illness. Due to the limited number of studies on the expression of FXR and CYP7A1 genes and their correlation with gallstone contents and demographic factors, this study aimed to quantitatively evaluate the expression levels of these genes in Iraqi female individuals with gallstone illness and to investigate the potential association between gene expression patterns and gallstone compositions as determined by FTIR spectroscopy.

METHODS

Study design and sample collection

A study including 52 female gallstone individuals who underwent cholecystectomy was undertaken at the Teaching Hospital Medical City and Al-Kadhimiya Teaching Hospital in Baghdad, Iraq, with ages spanning from 20 to 70 years, throughout February and April 2024. Two types of samples were taken, blood and gallstones, from all the individuals. Furthermore, age, white blood cell count, platelet count, and abdominal ultrasonography were incorporated into the case history. Male participants were excluded from the study. Furthermore, 50 controls were chosen to correspond with the individuals' ages. Control blood samples were procured from Majeed Private Hospital in Baghdad, Iraq.

Analysis of gallstones

Gallstones are taken from individuals via qualified surgeons utilizing laparoscopic cholecystectomy. The collected gallstones were positioned on sterile gauze to air dry, thereafter washed gently with doubly distilled deionized water to eliminate bile and debris, and dried on silica gel before being deposited into a tube labeled with the patient's name and the date [17]. Stone specimens were initially analyzed for their shape and color, and a digital photograph was captured of each item. They were categorized as cholesterol stones, black or brown-colored stones, and mixed stones [18,19]. Stones were bisected using a razor blade and subsequently crushed using a

pestle and mortar. The resultant sample was blended with spectral-grade KBr powder and finely processed to produce a 13 mm diameter disc in silver dies under 5 tons of pressure. The measurements of FTIR have been performed with a SHIMADZU IR-Prestige 21 FTIR spectrometer within the frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} [20].

Blood sampling and molecular analysis

A total of 3 ml of peripheral whole blood was aspirated from each participant straight into a tube containing EDTA. The blood samples were preserved using Trizol. The RNA was extracted from the blood specimens utilizing TRIzolTM (Thermo Scientific, UK). A 0.25 ml sample of blood from both individuals and controls was combined with 0.75 ml of TRIzol. Manufacturer's instructions were adhered to. The concentration and purity of the isolated RNA were evaluated using a NanoDrop spectrophotometer 2000c (Thermo Fisher Scientific, USA) through absorbance measurements at 260 and 280 nanometers. RNA samples showed an A260/A280 ratio between 1.8 and 2.0, which were determined to be acceptable for further study. EasyScript® First-Strand cDNA Synthesis SuperMix Kit (Transgene, China) was applied for reverse transcription of total RNA into complementary DNA (cDNA). The entire procedure was conducted in accordance with the manufacturer's specifications. The RNA-to-cDNA synthesis was performed in a thermal cycler using the following program: the reaction mixture was first subjected to incubation for 10 min at 25 °C, then for 15 min at 42 °C to allow reverse transcription, and finally heated at 85 °C for 5 min to terminate the reaction and inactivate the enzyme. The cDNA sequences of the *FXR (NR1H4), CYP7A1, and RPLP0* genes were acquired from the NCBI GenBank database. RT-qPCR primers were created in this work utilizing Primer 3 software. The primers were developed and employed according to the manufacturer's requirements (Macrogen, South Korea) (Table 1).

Table 1: The sequences and details of the primers applied in this study

		Primer sequences 5′→3′	GC (%)	Tm (˚C)	Product size (pb)
RPLP0	F	TGGTCATCCAGCAGGTGTTCGA	55	66.1	118
RPLP0	R	ACAGACACTGGCAACATTGCGG	55	64.5	
FXR (NR1H4)	F	CCAGGGAGAAACTGAGGTAGC	57	63.3	78
FXR (NR1H4)	R	CCTTTGATCCTCCCTGCTGA	55	63.1	
CYP7A1	F	CTGTGTGTCCCGCCTTGTAA	55	62.5	117
CYP7A1	R	AACTCAAAGACCGCTGCCTG	55	62.5	

Following cDNA synthesis, quantitative real-time PCR (qRT-PCR) was carried out using gene-specific primers to assess the expression levels of *FXR (NR1H4)* and *CYP7A1,* while *the RPLP0* gene served as an endogenous reference for standardization due to its stability. Oligonucleotide primers applied in the study were freeze-dried and purchased from Macrogen in South Korea. Reconstitution of the primer was done using nuclease-free water. For preparing a 10 pmol/µl working solution, 10 µl of the stored primer stock solution was diluted with 90 µl of nuclease-free water. For preparing a 100 pmol/µl primer stock solution, the primer was reconstituted using nuclease-free water. The methodology incorporated cDNA samples from both the patient and the control in a single run. Each sample employed three PCR tubes: one for the *FXR* experiment, another for *CYP7A1*, and the last tube for *RPLP0*, which is the housekeeping gene. The CyberGreen fluorescence method was applied in this investigation. A specified procedure was utilized to amplify cDNA samples via qPCR reactions. To attain a final volume of 20 µL, the subsequent components were incorporated: 10 µL of 2× Luna Universal qPCR Master Mix (Tinzyme, China), 0.5 µL (0.1 µM) of each primer, 3.5 µL of cDNA sample, and nuclease-free water. The qPCR program included one denaturing cycle at 95°C for 1 minute during pre-incubation, followed via 45 amplification cycles. Each amplification cycle included denaturation for 15 seconds at 95°C and annealing for 30 seconds at 60°C and extension for 30 seconds at 72°C. Finally, one final cycle was added for the melt range of 60-95°C for 40 min. The threshold cycle for each sample (CT) was estimated utilizing real-time cycler software, and mean results were produced. Selected genes' expression data were normalized using housekeeping. Using the 2-ΔΔCt method (Livak and Schmittgen) [21]. The ΔCt difference between housekeeping and target gene CT values was calculated for each sample as follows:

ΔCt Control = CT Targeted Gene - CTHKG.

ΔCt Patient = CT Targeted Gene – CT HKG

The difference in ΔCt values (ΔΔCt) was calculated as follows:

ΔΔCt = ΔCt (patient) - ΔCt (control)

Calculated gene expression fold change using this method: 2-ΔΔCt fold change.

Ethical considerations

The Ethics Committee of the Science College at the University of Baghdad granted ethical approval for this study with a reference number CSEC/0225/0013.

Statistical analysis

Qualitative data were expressed as mean ± standard deviation and/or number (%). Quantitative data (gene

expression) was expressed as median and interquartile range (IQR: 25–75%) using the 2-ΔΔCt method according to a normality test that showed nonparametric distribution. Significance was assessed using the Kruskal-Wallis test (for the comparison between more than two groups) or the Mann-Whitney U test (for the comparison between two groups). A probability *p-value* < 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics 27.0 software (Armonk, NY: IBM Corp.) and GraphPad Prism software version 9.2.0 (San Diego, CA, USA).

RESULTS

The baseline characteristics showed several significant differences between gallstone individuals and healthy controls (Table 2).

Table 2: Baseline characteristics and labertory data of individuals and healthy controls

Characteristics		Individuals (n=52)	HC (n=50)	*p*-value
Age (year)		41±14	37±14	0.181
Age group (year)	20-39	25(48.1)	34(68)	0.042
	40-75	27(51.9)	16(32)	
F.H	Yes	29(55.8)	0(0.0)	<0.001
	No	23(44.2)	50(100)	
Height; cm		158±7.0	162±6.0	0.016
Weight; kg		75±13	68±12	0.005
BMI; kg/m2		29.8±4.3	26.0±4.7	<0.001
	Normal (19-25)	5(9.6)	24(48)	<0.001
	Abnormal (> 25)	47(90.4)	26 (52.0)	
WBC; × 10^9/L		7.95±1.95	6.26±1.48	0.001
	< 4.5	1(1.9)	4(8.0)	
	4.5-12.0	48(92.3)	46(92.0)	0.032
	> 12.0	3(5.8)	0 (0.0)	
RBC × 10^9/L		4.64± 0.42	4.32±0.41	<0.001
	Normal (12-16)	48(92.3)	36(72)	0.007
	Abnormal	4(7.7)	14(28)	
HG × 10^9/L		12.53±1.51	11.32±1.68	<0.001
	Normal (4-5.5)	36 (69.2)	18(36)	<0.001
	Abnormal	16(30.8)	32(64)	
Platelets × 10^9/L		307.5±86.6	249.9±65.2	<0.001
	< 150	0(0.0)	2(4.0)	0.148
	> 150	52(100)	48(96)	
Medication	Untreated	7(13.5)	NA	NA
	Treated	45(86.5)		
Gallstone size (mm)		9.9±6.4		
	Low risk (< 5)	14(26.9)	NA	NA
	High risk (>5)	38(73.1)		
Gallstone type*	Cholesterol	20(38.5)		
	Pigment (black)	10(19.2)	NA	NA
	Mixed	22(42.3)		

Values are presented as frequency, percentage, and mean±SD. HC: Healthy controls; WBC: White blood cell count; HG: Hemoglobin; RBC: Red Blood Cell; BMI: Body Mass Index; F.H: Family History; NA: Not Detectable. Significance was assessed using one-way analysis of variance test or Pearson's Chi-square test (categorical variable).

Although the mean age didn't show significant differences between groups (p= 0.181), the distribution of age categories showed a higher proportion of younger individuals (20–39 years) among controls (p= 0.042). Family history was markedly more frequent in individuals (55.8%) and absent in controls (p< 0.001). Individuals exhibited lower height and higher body weight compared to controls, which led to a significantly elevated BMI (p< 0.001). The hematological analysis had higher WBC, RBC, RBC, hemoglobin, and platelet counts in individuals, with most of these differences being significant. Only the patient group had additional clinical characteristics identified, such as gallstone size, risk classification, and stone type. The expression levels of *FXR* and *CYP7A1* genes were significantly reduced in gallstone patients compared with healthy controls (Figure 1).

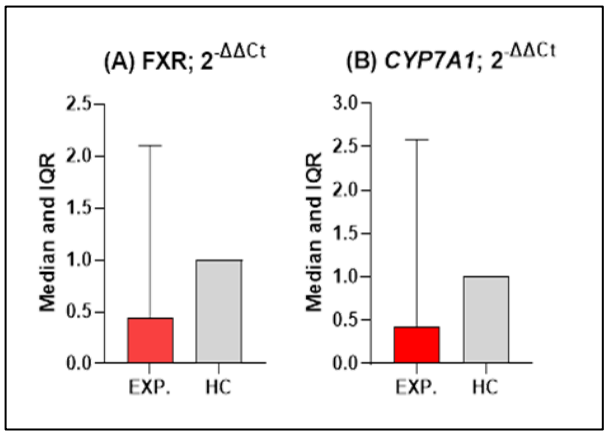

Figure 1: *FXR* and *CYP7A1* gene expression levels in gallstone individuals expressed as median and interquartile range. Expression values in healthy controls were normalized to 1.

The values of the control group were normalized to 1.0. When gene expression was stratified via clinical characteristics, no significant associations were observed with age, BMI, hematological indices, treatment status, gallstone size, or stone type (p> 0.05 for all). In contrast, family history was significantly associated with reduced expression of both *FXR* (p= 0.004) and *CYP7A1* (p= 0.003) (Table 3).

Table 3: The expression level of *FXR* and *CYP7A1* genes (fold change; $2^{-\Delta\Delta Ct}$) stratified via clinical characteristics of gallstone individuals

Characteristic		*FXR* expression; $2^{-\Delta\Delta Ct}$		*CYP7A1* expression; $2^{-\Delta\Delta Ct}$	
		Median	IQR 25–75	Median	IQR 25–75
Individuals	All	0.448	0.066- 2.045	0.421	0.058- 2.488
Age group; years	20-40	0.152	0.061- 1.102	0.196	0.039- 0.994
	40-75	0.525	0.095- 2.584	0.467	0 .076- 4.003
	p-value	0.301		0.145	
F.H	Yes	0.107	0.042- 0.864	0.109	0.027- 0.847
	No	0.774	0.403- 2.657	0.587	0 .387- 4.003
	p-value	0.004		0.003	
BMI; kg/m2	Normal (19-25)	0.107	0.044- 0.774	0.109	0.012- 0.587
	Abnormal (> 25)	0.493	0.069- 2.158	0.445	0.063- 3.343
	p-value	0.449		0.316	
WBC; × 10^9/L	< 4.5	0.072	0.072- 0.072	0.076	0.076- 0.076
	4.5-12.0	0.448	0.062- 2.173	0.421	0.049- 3.010
	> 12.0	0.717	0.107- 1.094	0.791	0.109- 2.299
	p-value	0.732		0.737	
RBC; × 10^9/L	Normal (12-16)	0.508	0.071- 2.172	0.444	0.058- 3.010
	Abnormal	0.172	0.020- 0.511	0.226	0.032- 1.343
	p-value	0.171		0.396	
HG; × 10^9/L	Normal (4-5.5)	0.448	0.077- 1.723	0.426	0.058- 3.657
	Abnormal	0.476	0.066- 2.044	0.421	0.051- 1.083
	p-value	0.812		0.383	
Platelets; × 10^9/L	< 150	0.0	0.0-0.0	0.0	0.0-0.0
	> 150	0.448	0.066-2.044	0.421	0.058-2.488
	p-value				
Medication	Untreated	0.524	0.125-4.855	0.877	0.129-4.534
	Treated	0.403	0.062-1.931	0.401	0.053-1.880
	p-value	0.528		0.255	
Gallstone Size; mm	Low risk (< 5)	1.210	0.060-2.927	0.647	0.065-3.893
	High risk (>5)	0.334	0.069-0.864	0.276	0.053-2.298
	p-value	0.143		0.409	
Gallstone type	Cholesterol	0.463	0.066-1.738	0.356	0.040-1.363
	Pigment (black)	0.256	0.060-1.093	0.259	0.039-3.657
	Mixed	0.559	0.071-2.584	0.468	0.076-4.002
	p-value	0.827		0.442	

WBC: White blood cell count; HG: Hemoglobin; RBC: Red Blood Cell; BMI: Body Mass Index; F.H: Family History; IQR: Interquartile range; NA: Not Detectable.

Correlation analysis revealed significant relationships between gene expression and selected clinical and laboratory parameters (Figure 2).

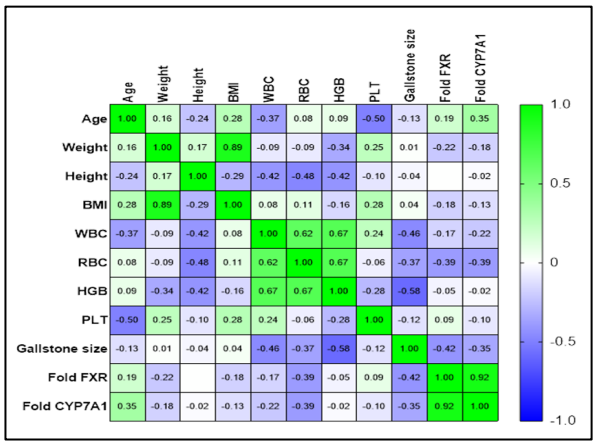

Figure 2: Correlation analysis between baseline laboratory data in individuals with gallstone. Values inside boxes indicate the correlation coefficient (rSp). The green color indicates a positive correlation while the blue color indicates a negative correlation.

FXR and *CYP7A1* expression levels showed a strong positive correlation, while both genes demonstrated inverse correlations with gallstone size and selected hematological parameters, including WBC, RBC, hemoglobin, and platelet counts. Gallstones obtained from individuals were classified based on physical appearance into cholesterol, pigment, and mixed types. Cholesterol gallstones appeared smooth and round with a pale brown or yellow color (Figure 3). Their FTIR spectra were characterized by the presence of broad OH and aliphatic stretching bands in addition to minor compounds but the absence of bilirubin (Figure 4). On the other hand, the pigment type showed dark and rough in texture (Figure 3). FTIR analysis exhibited major absorption patterns of typical calcium bilirubinate and carbonyl and amide-related peaks with limited carbonate band presence (Figure 4). Mixed gallstones, which can be considered the most abundant type (Figure 3), exhibited a morphology that fell between the two other patterns, and their FTIR spectra revealed this as well, in which the

cholesterol band appeared alongside those related to bilirubinate without the dominance of any component.

Figure 3: The three types of gallstones, 1: cholesterol, 2: Pigment, and 3: mixed.

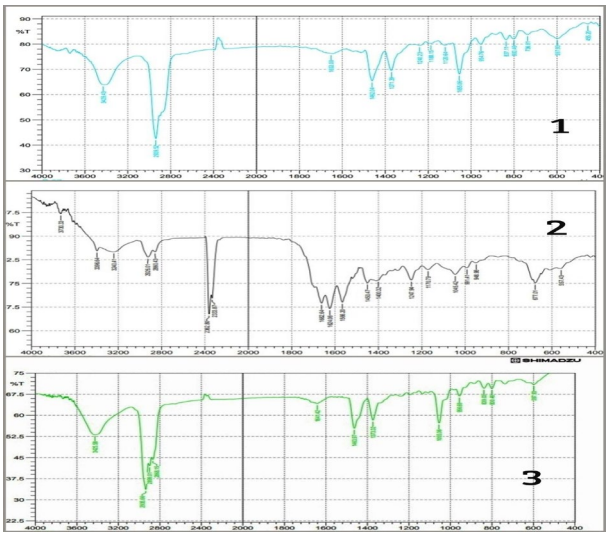

Figure 4: FTIR spectrum of 1) cholesterol, 2) pigment, and 3) mixed gallstone.

DISCUSSION

The present study explored the relationship between clinical characteristics, gallstone composition, and the expression of bile acid–related genes in individuals with gallstone illness. Several findings from this work point toward a multifactorial nature of the illness, in which metabolic, genetic, and biochemical factors appear to interact rather than act independently [22]. In line with previous research, gallstone individuals in this study differed from healthy controls in terms of body mass index and hematological parameters, as elevated platelet counts and white blood cells reflect the systemic inflammatory state associated with metabolic dysregulation and obesity, which in turn contributes to gallstone formation via impairing gallbladder motility and promoting oxidative stress [23,24]. The increase in obesity will cause an increase in the rate of cholesterol synthesis in the liver, resulting in cholesterol supersaturation and bile stasis [25]. This finding is also in support of the view that gallstone illness is associated with metabolic imbalance and low-grade inflammation [8,23]. The presence of positive history among the individuals suffering from gallstone illness could indicate that inherited factors could be a risk for the development of gallstone illness, apart from other factors [26]. At the genetic level, it was found that the expression rate of *FXR* and *CYP7A1* was lower in the individuals than that of healthy individuals. The reduced expression of FXR may also be of significance in other functions besides bile acid regulation. FXR is known to be involved in the regulation of bile acids [27]; it is involved in the regulation of bile acid synthesis, transport, and enterohepatic circulation. Under physiological conditions, activation of *FXR* inhibits transcription of the *CYP7A1* gene through a negative feedback mechanism. However, as demonstrated in this study, the reduction of *FXR* and *CYP7A1* expression suggests that this tightly regulated pathway could be disrupted, which would result in a change in bile acid pool size or composition, thereby changing the solubility of cholesterol in bile. Altered *FXR* expression has been found to result in reduced expression of bile salt export proteins and lipid metabolism, which may result in cholesterol supersaturation, an important step in gallstone formation [28]. Likewise, the importance of the enzyme *CYP7A1*, which is the rate-limiting enzyme in the classical pathway of bile acid biosynthesis, cannot be overstressed in the maintenance of cholesterol homeostasis. Any decrease in the expression of this enzyme may result in the reduction of cholesterol converted to bile acids, causing an increase in cholesterol in the bile. This could perhaps lead to an increase in nucleation and crystal formation, but in individuals who are already more likely to do so for a variety of reasons related to their metabolism and genetics. The positive correlation between *FXR* and *CYP7A1* suggests that these two genes are interdependent. It can therefore be stated that the above findings reinforce the fact that a change in the *FXR/CYP7A1* pathway could perhaps be a key factor that links the genetic predisposition to the biochemical changes in bile. Rather than acting as isolated risk factors, changes in these genes may reflect an underlying disturbance in hepatic metabolic balance, which, when combined with environmental and lifestyle factors, promotes gallstone formation [29]. Such value can be increased if their evaluation is performed along with other chemical or clinical indicators. Via using FTIR spectroscopy and determining gallstone composition, more information has been obtained on the variability of human illness [30]. These components, such as cholesterol, pigments, and mixed gallstones, suggest that the process of stone formation does not occur in one singular fashion. The fact that several components of mixed gallstones were collected in this study, together with the analysis of the genes expressed, implies that bile

acids are not just involved in the process of stone formation but are also involved in the process of pigments. This work makes it easy to understand the composition of several gallstones and how they are all mixed up, rather than being in one category [22]. The results suggest that gallstone illnesses are not likely to occur as a result of the effect of one cause, but they occur as a result of the effect of all the factors, such as genetic factors, metabolic changes, and changes in the composition of bile [31,32]. The rationale for conducting the study is grounded in the molecular basis and composition of gallstones; it is important to note that the results should be interpreted within the context of a cross-sectional study approach. The study can be extended to a wider population sample, and the results obtained can be applied in conjunction with a functional study on the importance of the composition of gallstones in relation to bile acids.

Study Limitations

This study has several limitations that should be considered. The sample size was relatively limited, which could affect the generalizability of the findings. Additionally, the study focused only on two bile acid–related genes (*FXR* and *CYP7A1*), while other regulatory genes involved in bile acid metabolism were not examined, which may have an important role in gallstone formation. Another limitation was the difficulty in obtaining clinical samples, particularly gallstone specimens and matched blood samples, due to the dependence on surgical procedures and patient consent, which restricted the number of eligible participants. Finally, the study population included only Iraqi female participants, which may limit the extrapolation of the findings to other populations.

Conclusions

The present study demonstrated a significant downregulation in the expression of *FXR* and *CYP7A1* genes in Iraqi female patients with gallstone disease compared with healthy controls. This finding suggests a potential disturbance in bile acid regulation that may promote gallstone formation. In addition, the prevalence of mixed gallstones identified by FTIR analysis and the high frequency of positive family history among patients demonstrate the multifactorial nature of gallstone disease. Overall, these results provide the possible role of bile acid–related genes in gallstone pathogenesis. So, this study may be the first of its kind in Iraq to investigate the expression profiles of *FXR* and *CYP7A1* genes in gallstone disease and their correlation with demographic factors and gallstone compositions.

Conflict of interests

The authors declared no conflict of interest.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Data can be shared with the corresponding author upon reasonable request, and subject to ethical approval.

REFERENCES

1. Sun H, Warren J, Yip J, Ji Y, Hao S, Han W, et al. Factors influencing gallstone formation: A review of the literature. *Biomolecules*. 2022;12(4):550. doi: 10.3390/biom12040550.
2. Pęczuła A, Czaplicki A, Przybyłkowski A. Genetics of gallstones. *Genes*. 2025;16(3):256. doi: 10.3390/genes16030256.
3. Khalaf SK, Al Mousawi J, Hussein A, Al Asadi J. Prevalence and risk factors of asymptomatic gallstones in a sample of population in Basrah, Iraq. *Arch Med*. 2016;8(4):1-6. doi: 10.21767/1989-5216.1000146.
4. Al-Obaidi SM, Abdulla TS, Al-Alawi MS, Mohammed HM. The prevalence of silent gallstones and its relation to some risk factors in Iraq. *Iraqi Postgrad Med J*. 2006;5(2):146-150.
5. Li H, Zhang C. Association between triglyceride-glucose index and gallstones: a cross-sectional study. *Sci Rep*. 2024;14(1):17778. doi: s41598-024-68841-6.
6. Zheng J, Dong H, Wan H, Yang Q, Xu S, Hu T, et al. Positive association between cardiometabolic index and gallstones, with greater impact on women and those younger than 50 years: the NHANES 2017–2020 cross-sectional study. *BMC Pub Health*. 2025;25(1):2130. doi: 10.1186/s12889-025-23323-w.
7. Simonsen MH, Erichsen R, Frøslev T, Rungby J, Sørensen HT. Postmenopausal estrogen therapy and risk of gallstone disease: a population-based case–control study. *Drug Saf*. 2013;36(12):1189-1197. doi: 10.1007/s40264-013-0100-5.
8. Parra-Landazury N, Cordova-Gallardo J, Méndez-Sánchez N. Obesity and gallstones. *Visc Med*. 2021;37(5):394–402. doi: 10.1159/000519148.
9. Lee G, Suh JY, Kim J, Park TY, Do JH, Choi YS, et al. Prevalence of cholesterol gallstones in a Korean population over a 14-year period. *Korean J Intern Med*. 2025;40(4):584-591. doi: 10.3904/kjim.2025.090.
10. 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):647-668. doi: 10.1007/s12551-020-00672-4.
11. Ali S, Rasul S, Dawani S, Zahid S, Hussain S, Sarwar O, et al. Effects of chemical composition of cholesterol and pigment stones on the gallbladder mucosa. *J Bahria Univ Med Dent Coll*. 2022;12(02):68-72.
12. Dowais R, Al Sharie S, Araydah M, Al Khasawneh S, Haddad F, AlJaiuossi A. Pearl-white gallstones: A report of a case and a chemical analysis via FTIR and XRD. *Int J Surg Case Rep*. 2021;87:106449. doi: 10.1016/j.ijscr.2021.106449.
13. Arrout A, El Ghallab Y, Hirri A, Aït Mouss R, Yamari I, Lefriyekh MR, et al. Prediction of cholesterol content in gallstones via FTIR spectroscopy coupled with chemometric tools. *Microchem J*. 2024;199:109956. doi: 10.1016/j.microc.2024.109956.
14. Wang Y, Xu H, Zhou X, Chen W, Zhou H. Dysregulated bile acid homeostasis: unveiling its role in metabolic diseases. *Med Rev*. 2024;4(4):262-283. doi: 10.1515/mr-2024-0020.
15. Chiang JY, Ferrell JM, Wu Y, Boehme S. Bile acid and cholesterol metabolism in atherosclerotic cardiovascular disease and therapy. *Cardiol Plus*. 2020;5(4):159-170. doi: 10.4103/2470-7511.305419.
16. Chiang JY, Ferrell JM. Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis. *Liver Res*. 2020;4(2):47-63. doi: 10.1016/j.livres.2020.02.003.
17. Sikkandar S, Jayakumar S, Gunasekaran S, Renugadevi T, Alwar B. Study on the analysis of human gallstones using Fourier transform infrared spectroscopic technique. *Int J ChemTech Res*. 2011;3(1):149-154.

18. Ha BJ, Park S. Classification of gallstones using Fourier-transform infrared spectroscopy and photography. *Biomaterials Res*. 2018;22(1):18. doi: 10.1186/s40824-018-0139-0.
19. Bilagi A, Godhi AS. An analytical study of gallstones via Fourier transform infrared spectroscopy technique. *Int Surg J*. 2022;9(3):584-589. doi: 10.18203/2349-2902.isj20220625.
20. Raman RG, Selvaraju R. FTIR spectroscopic analysis of human gallstones. *Rom J Biophys*. 2008;18(4):309-316.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*. 2001;25(4):402-408. doi: 10.1006/meth.2001.1262.
22. Portincasa P, Di Ciaula A, Bonfrate L, Stella A, Garruti G, Lamont JT. Metabolic dysfunction-associated gallstone disease: expecting more from critical care manifestations. *Intern Emergency Med*. 2023;18(7):1897-1918. doi: 10.1007/s11739-023-03185-5.
23. Fu C, Chen J, Wang Y, Yang Y, Li X, Liu K. Association between complete blood cell count-derived inflammatory biomarkers and gallstones prevalence in American adults under 60 years of age. *Front Immunol*. 2025;15:1497068. doi: 10.3389/fimmu.2025.1497068.
24. Parra-Landazury NM, Cordova-Gallardo J, Méndez-Sánchez N. Obesity and gallstones. *Visc Med*. 2021;37(5):394-402. doi: 10.1159/000519148.
25. Wang K, Liu Z, Tang R, Sha Y, Wang Z, Chen Y, et al. Gallstones in the era of metabolic syndrome: pathophysiology, risk prediction, and management. *Cureus*. 2025;17(3). doi: 10.7759/cureus.80541.
26. Costa CJ, Nguyen MTT, Vaziri H, Wu GY. Genetics of gallstone disease and their clinical significance: A narrative review. *J Clin Transl Hepatol*. 2024;12(3):316. doi: 10.14218/JCTH.2023.00563.
27. Fuchs CD, Simbrunner B, Baumgartner M, Campbell C, Reiberger T, Trauner M. Bile acid metabolism and signalling in liver disease. *J Hepatol*. 2025;82(1):134-153. doi: 10.1016/j.jhep.2024.09.012.
28. Ye X, Huang D, Dong Z, Wang X, Ning M, Xia J, et al. FXR signaling-mediated bile acid metabolism is critical for alleviation of cholesterol gallstones via lactobacillus strains. *Microbiol Spectrum*. 2022;10(5):e00518-22. doi: 10.1128/spectrum.00518-22.
29. Li X, Yin X, Xu J, Geng L, Liu Z. Relationship between abnormal lipid metabolism and gallstone formation. *Korean J Gastroenterol*. 2025;85(1):11-21. doi: 10.4166/kjg.2024.135.
30. Shaltout AA, Seoudi R, Almalawi DR, Abdellatief M, Tanthanuch W. Quantitative phase analysis and molecular structure of human gallstones using synchrotron radiation X-ray diffraction and FTIR spectroscopy. *Spectrochim Acta Part A Mol Biomol Spectroscopy*. 2024;308:123777. doi: 10.1016/j.saa.2024.123777.
31. Higashizono K, Nakatani E, Hawke P, Fujimoto S, Oba N. Risk factors for gallstone disease onset in Japan: Findings from the Shizuoka Study, a population-based cohort study. *Plos One*. 2022;17(12):e0274659. doi: 10.1371/journal.pone.0274659.
32. Ravi PC, Thugu TR, Singh J, Dasireddy RR, Kumar SA, Isaac NV, et al. Gallstone disease and its correlation with thyroid disorders: a narrative review. *Cureus*. 2023;15(9). doi: 10.7759/cureus.45116.