

Predicting the chemical composition of Gallstones by FTIR spectroscopy

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ABSTRACT

Gallstones represent a prevalent and costly health problem. Chemical composition of gallstone is essential for aetiopathogenesis of gallstone diseases. This study aimed to define patterns of gallstone composition in 162 samples surgically removed from as many patients 144 females and 18 males, age range 15 to 75 years, admitted for treatment in Rizgary Teaching Hospital, Kurdistan, Iraq, during 2007-2008. All stones were analyzed by Fourier transform Infrared (FTIR) Spectroscopy for cholesterol, bilirubin and calcium carbonate. Of the 162 samples of gallstones analyzed, 108 (66.7%) were identified as pure cholesterol stones, 52 (32.1%) were mixture of cholesterol + bilirubin and only 2 (1.2 %) were identified as calcium bilirubinate. Gender wise comparisons revealed that majority of gallstones were recovered from the female patients. We also concluded that, incidence of gallstone was higher in age group ≥ 45 years.

Key words: Gallstone, FTIR, chemical composition, mixed gallstone, cholesterol, bilirubin, calcium carbonate.

INTRODUCTION

Gallstones represent a serious burden health care systems: 10-20% of Europeans and Americans carry gallbladder stones^{1,2}. The prevalence of gallstones disease is rising, possibly as a result of longer life expectancy and altered nutritional habits.

In particular, the increase of life style related risk factors was assumed to result primarily in an increase of cholesterol gallstones³. The impact of life style and ethnicity is underscored by the profoundly different gallstone composition in recent studies from sub-Saharan Africa⁴ and China^{5,6}. Many gallstones are silent, but symptoms and complications ensue in around 25-50% of cases. The identification of the components of gallstones is essential as it provides information that could be useful for practitioners to find out the underlying

cause of gallstones and to decide whether to treat gallstone patients therapeutically or surgically^{7,8}.

According to literature data, cholesterol, and bilirubin. Bile and fatty acids and their calcium salt and inorganic compounds (mainly calcium carbonate) are the general components of all gallstones^{9,11}, in addition to major, minor and trace elements namely S, Cl, K, Co, Ti, Ca, V, Cr, Mn, Fe, Ni, Cu, Zn, Br, Fe and Pb¹²⁻¹⁶. An abnormal case of a premature neonate who developed calcium oxalate-phosphate gallstones detected at four months of age¹⁷. The application of novel preventive of therapeutic non-surgical approaches will likely limited to certain classes of gallstones as defined by their composition and etiology. The pathogenesis of gallstones is not clearly understood¹⁸. Analysis of the chemical composition of gallstones offers clues to the pathogenesis of gallstones. Chemical analysis of gallstones was done by FTIR

spectroscopy, a technique requiring neither crystallinity nor volatilization to quantitate cholesterol, bilirubin and calcium carbonate¹⁹. No study of the chemical analysis of gallstone from patients in Erbil has been carried out to date, the present study was conducted to describe the chemical composition of gallstones recovered from patients in our area.

MATERIAL AND METHODS

Gallstones from 162 patients of cholelithiasis were collected after cholecystectomy at local hospital (Rizgary Teaching Hospital) during 2007-2008. In all, 162 gallstones were collected from 144 female and 18 males, age range 20-75 years. The stones were divided into 3 groups⁽²⁰⁾ depending upon their color pale yellow and whitish stones as cholesterol calculi, black and blackish brown as pigment calculi brownish yellow or greenish with laminated features as mixed calculi. The other relevant information's about the patients such as age, sex, number of calculi and the date of operation were obtained from hospital records. All gallstones removed during surgery were placed on sterile gauze to air dry and then washed carefully with doubly distilled deionized water (to removed bile and debris) and dried over silica gel for at least seven days.

After noting the morphological features such as color and shape, single gall stone from each patient (heaviest one in case of multiples) was cut into quarters using a jeweler saw (to obtain representative samples from center and periphery) and one quarter was ground separately with pestle and mortar for 5 minutes. This process produced a fine homogeneous powder. Then stored in a sample tube, kept over silica gel in dark cabinet until analyzed for composition.

One to two milligrams of the resulting powder were mixed with 300 milligrams of potassium bromide and pressed with eight to nine tons (corresponding to 0.7-1 Gpa) to generate KBr disk with 13 mm diameter⁷. These slices were measured using FTIR spectrometer on a FTS, IR 300 spectrometer thermo, Mattson, (095-3490) in the range of 400-4000 cm⁻¹ resolution .

Control spectra from the manufactures library were used for comparison with pure cholesterol, bilirubin and calcium carbonate standard. Two samples for each stone composition were analyzed. The composition of the central, the periphery and the whole stone powder for cholesterol gallstones were determined.

RESULTS

By the help of chemical composition of gallstones, proper management of the disease and prevention of its reoccurrence is possible. Typical FTIR spectra of three different standards, cholesterol, bilirubin and calcium carbonate are shown in figures 1 to 3, then compared with the typical FTIR spectra of gallstone samples. Figures 4 to 6 show the typical FTIR spectra of the selected gallstone samples, which were e^{90%} matched with the spectra of the standards and identified.

Table 1 presents the type of gallstone identified, their occurrence and presentage. Out of 162 gallstones 108 (66.7%) were identified pure cholesterol, 52 (32.1%) were mixed stones and only 2 (1.2%) were identified as pigment stone. All the mixed component gallstones were of cholesterol + bilirubin type.

Table 2 presents IR bands of principle components observed in the studied gallstones. The diagnostic bands identified for cholesterol were the strong bands around 3438, 2935, 1465 and 1056 cm⁻¹ while the diagnostic band for bilirubin and calcium bilirubin were 1633, 1565, and 1275, 1661, 1249, 1397, and 1435 cm⁻¹.

Table 1: Types, number and percentage of stones recovered from patients

Percentage	No. of stone	Type of stones
Pure cholesterol	108	66.7
Cholesterol + Bilirubin	52	32.1
Calcium bilirubinate	2	1.2
Pure calcium carbonate	-	-
Cholesterol + Calcium carbonate	-	-

Table 2: Types of gallstones identified and IR bands of principal components observed in analyzed gallstones

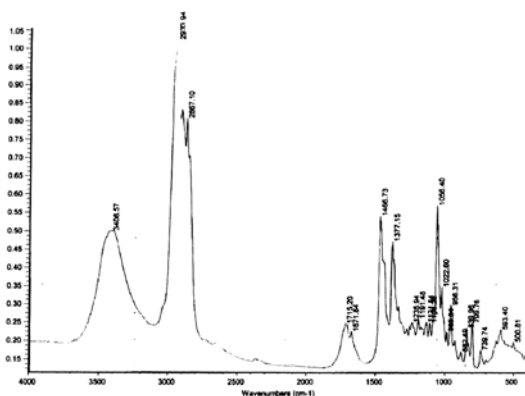
S. No.	Gallstones	Principal IR observed bands
1.	Pure cholesterol	2958.43, 2899, 2866.6, 1466.24, 1056.56
2.	Cholesterol + Bilirubin	The same bands correspondin to pure cholesterol and 1670.19 1636.2
3.	Calcium bilirubiante	3399.85 1661.93 139.96
4.	Pure calcium carbonate	-
5.	Cholesterol + Calcium carbonate	-

Table 3: Gender-wise comparison of stones recovered from patients

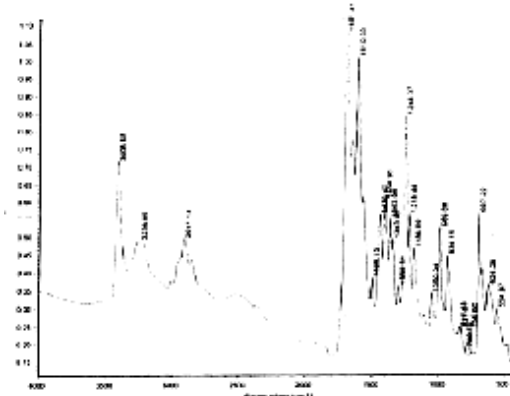
Gender	No. of stone	Percentage
Female	144	88.9
Male	18	11.1

Table 4: Gender-wise comparison of stones recovered from patients

Age/Years	No. of patients	Percentage
25-29	31	19.1
30-44	48	29.6
45 and above	83	51.2

**Fig. 1: Typical FTIR spectrum of pure cholesterol(standard)**

Comparison of composition of different parts of gallstones (central and the peripheral parts) with the whole stone powder, are shown in figures 7, 8 and 4 respectively. The results revealed that cholesterol was present in high concentration in all parts of the studied cholesterol gallstones. Data in table 3 revealed that majoring (88.9%) of gallstones were recovered from the female patients. Informations in table 4 disclosed that majority of stones (51.2%) were recovered from the patients of age ≥ 45 years.

**Fig. 2: Typical FTIR spectrum of pure bilirubin(standard).**

DISCUSSION

Pure cholesterol gallstone were characterized by the bands around 2958.43, 1466.24 and 1056.56 cm^{-1} cholesterol in mixed composition stones was characterized by the bands between 2800-3000 cm^{-1} due to asymmetric and symmetric stretching vibrations of CH_2 and CH_3 groups as far as pure cholesterol gallstones were concerned, some had a radial structure from the center to the periphery on cross section. Whereas

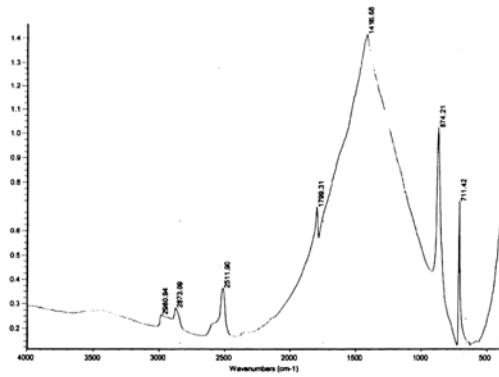


Fig. 3: Typical FTIR spectrum of pure calcium carbonate(standard)

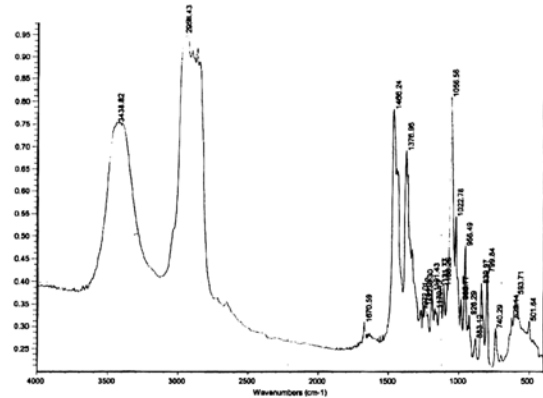


Fig. 4: Typical FTIR spectrum of cholesterol gallstone samples

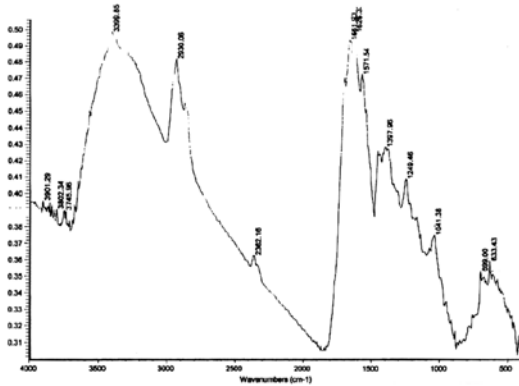


Fig. 5: Typical FTIR spectrum of Cholesterol + Bilirubin gallstone samples

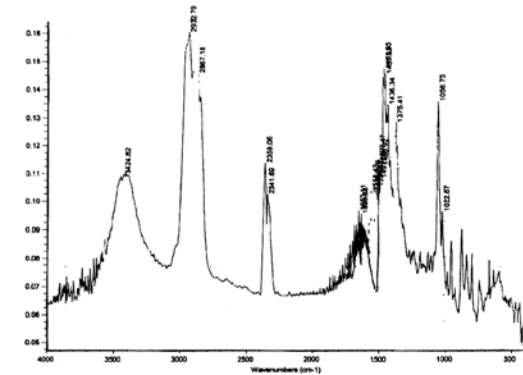


Fig. 6: Typical FTIR spectrum Calcium bilirubiante gallstone samples

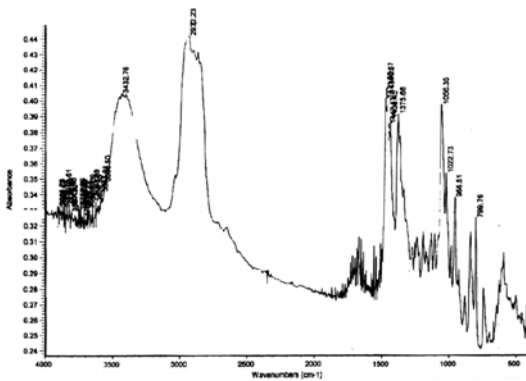


Fig. 7 : Typical FTIR spectrum of a central part cholesterol of gallstone samples

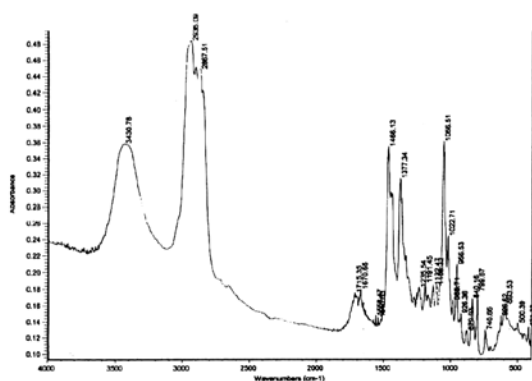


Fig. 8: Typical FTIR spectrum of a periferal part of cholesterol gallstone samples

most had pigment at the center and grossly visible cholesterol crystals at periphery. Comparison of composition of different parts of gallstones, central part, the periphery (Figs. 7,8) and the whole stone (Fig. 4) revealed that, concentration of cholesterol was markedly high in the periphery, center and in the whole stone powder.

Data in tables 1 and 2 revealed that most prevalent substance was cholesterol which was detected in 95% of the studied gallstone samples. Thus cholesterol gallstone represents the dominating type of gallstone. Our observation is in agreement with those observed in study from Northern German¹⁸ and in contrast to that from sub-Saharan Africa⁴.

The characteristics band features and key band locations (Table 2) for the components of gallstones in the present study were agreement with those reported in literatures^{21,22}. The cholesterol gallstone disease is indeed a multifactorial disease. The important epidemiological risk factors for cholesterol gallstone formation include diabetes mellitus²³, physiological stress²⁴, parity²⁵ and diet low in calcium²⁶. Additional factors associated with cholesterol gallstone disease are sedentary life styl, obesity, aging, femininity, diet rich in saturated fats, simple sugars and other dietary factors like consumption of rapeseed oil, cotton seed oil, butter, beans, tomatoes and tea. Although, it is generally a greed that gallstone composition mainly depends upon dietary habits of the patients, there is still little agreement about the risk of specific dietary components for specific type of gallstone²⁷. Gender wise comparison revealed that out of 162 gallstone patients, 144 were females and only 18 were males

and thus the females to male ratio was 8:1, which was in accordance with literatures, Rains²⁸ advocated that concentration of bile salts in bile is reduced by oestrogen and there by making it lithogenic. Horn²⁹ postulated that under the influence of female sex hormones, the muscle may relax, billiary passage dilates and duodenal content of pancreatic secretion regurgitates into gallbladder and promote conditions which favor the formation of gallstones. Cuevas *et al*³⁰ concluded that females had a greater risk of gallstone diseases, especially if they had used oral contraception and /or had four or more children.

Out of the total number of gallstones collected, the incidence of gallstones was higher in age group 45 years and above (Table 4). Our observations are in conformity with with earlier study from Hayana⁸ and also reports from Assam² and Aligarh³.

CONCLUSION

Analysis of gallstones based on FTIR revealed that cholesterol either singly or in combination with either bilirubin or calcium carbonate is the most predominant component of gallstones in Erbil, Kurdistan, Iraq. The concentration of cholesterol was markedly high, both in the periphery and center of the gallstones, Gender wise comparison revealed that majority of gallstones were recovered from the female patients. We also concluded that, incidence of gallstone was higher in age group e" 45 years. Our study suggests that cholesterol and bilirubin are the main risk factors of gallstone diseases.

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