Identification of Chemical Component of Gallstones using FT-IR Spectroscopy, SEM and EDS in Al-Nasiriya City, South of Iraq

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Abstract
This study focus on the identification the chemical component of gallstones obtained from 66 patients, 57female and 9 male. Range 19 to 55 years (2 patients above 70), the gallstones were removed during surgical operations at Al-hussain hospital (Al-Nasiriya city) using FTIR spectroscopic method. The identification shows four main kinds of gallstones found in patients, were identified as pure cholesterol (30.30%), pure calcium bilirubinate(15.15%), cholesterol mixed with calcium bilirubinate (47%) and cholesterol mixed with calcium carbonate(7.57%) gallstones. Incidence of gallstones was higher in age between 20 and 50 years. The majority of gallstones were removed from the female than male 6.33:1. Also, in this work, Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) were applied for a study of the morphological structure and determination of major and trace element composition of gallstone samples.
Keywords: gallstones, FTIR spectroscopy, SEM, EDS

1. Introduction

Gallstone is a common digestive disease, formation in the gallbladder, bile duct and liver [1]. There are three kinds of gallstones found in patients, white, black and brown. The main component of white stones (sometime yellow in color) is cholesterol.
Brown stones are usually contain calcium bilirubinate and black stones typically form in gallbladder result when excess bilirubin enters the bile and polymerizes into calcium bilirubinate [2].
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 gallstone and to decide whether to treat
Gallstone patients therapeutically or surgically. Unfortunately, gallstone composition is hetero-generous, and varies within and amongst the populations around the world [2].
The prevalence of gallstone disease is rising for more reasons, longer life expectancy and altered nutritional habits, fat, fertile, flatulent ,female of 50 years and people who have cirrhosis, biliary tract infection and hereditary blood disorders such as sickle cell anemia ,Diets high in cholesterol and fat [3].

2. Materials And Methods

Sixty six gallstones, collected from gallbladders of patients after surgeons using cholecystectomy surgery, were provided by Al-hussain hospital. 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. After noting the morphological features such as color and shape, 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.
The gallstones from each patient was ground separately with pestle and mortar. This process produced a fine homogeneous powder. Then stored in a sample tube, kept over silica gel in dark cabinet until analyzed for composition. Few milligrams of the resulting powder were mixed convenient milligrams of potassium bromide and pressed to generate KBr disk with. These slices were measured using FT-IR spectrometer on in the range of 400-4000 cm-1 resolution. The gallstones were classified by comparing the respective absorption peaks with the values reported in the literature. The SEM micrographs and the elemental composition of GS samples were taken by using SEM, EDS (LUM TGSCAN BRNO-mir3 Czech Republic)

3. Results and Discussion

The type of gallstones identified by FTIR spectra is presented in table 1. Their occurrence and percentage, out of 66 gallstones 20(30.30%) were identified pure cholesterol, 10(15.15%) pure calcium bilirubinate, 31(46.96%) cholesterol Mixed with calcium bilirubinate, 5(7.57%) cholesterol mixed with calcium carbonate.

Table 1: Type, number and percentage of stones from 66 patients :

Type of stones	No.of stones	Percentage%
Pure cholesterol	20	30.30
Pure calcium bilibrubinate	10	15.15

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Cholesterol + calcium bilirubinate	31	46.96
Cholesterol + calcium carbonate	5	7.57

Table 2: patient's distribution according to gender		
six	No. of patients	Percentage %
female	57	86.36
meal	9	13.64

Table 3: percent distribution of patients according to age		
Age	Number	Percentage %
10-19	1	1.5
20-29	15	22.73
30-39	20	30.30
40-49	21	31.81
50-59	7	10.60
60-69	-	-
70-79	2	3.03

Pure cholesterol gallstones

The FT-IR spectrum of a pure cholesterol gallstone is shown in Fig. 1, the absorption band at 2935.61 cm-1 due to asymmetric stretching of CH2, a CH2 symmetric stretching absorption band at 2868.70 cm-1, a CH2 symmetric bending absorption band at
1374.57 cm-1, and a C-C stretching absorption band at 1056.58 cm-1 [4]. A broad OH stretching absorption band appears at 3397.12 cm-1.

Calcium billrubinte gallstones

triplet bands at (1663.00, 1626.39 and 1570.33) cm-1, The first two bands were observed for bilirubin, and the 1570.33 cm-1 band was observed only when the carboxylic acid of bilirubin is conjugated with metal ion such as calcium, forming calcium bilirubin [5].a shoulder band appears at 1699.37 cm-1 was assigned to vibration of a non-conjugated carboxylic acid [5, 6]. The absorption bands at 1442.56 cm-1 were assigned to the pyrrole ring deformation and was observed for the FT-IR spectrum of a calcium bilirubinate gallstone is shown in Fig. 2.The spectrum had characteristic doublet absorption band at 3394.88 cm-1 and 3233.51 cm-1. One of the doublet at 3394.88 cm-1 is sharp was assigned as a N-H stretching vibration of the pyrrole groups, and the broad band at 3233.51 cm-1 as the lactam N-H stretching vibration [7]. The spectrum also shows both bilirubin and calcium bilirubin, The absorption band at 1247.03 cm-1 was assigned for amino C-N/carboxylate C-O stretching, and at 699.16 cm-1 for the lactam ring deformation .The spectrum matched well with the FT-IR spectrum of pure calcium bilirubinate reported by [5, 8].

3-Cholesterol Gallstones mixed with calcium bilirubinate

Fig.3, shows the FT-IR spectrum of cholesterol gallstones mixed with calcium bilirubinate. The spectrum consisted of the characteristic absorption band of calcium bilirubinate at (3393.62. 2933.93, 1666.77, at (2936.86, 1373.85, and 1055.16) cm-1.

4-Cholesterol gallstones mixed with calcium carbonate

As shown in Fig. 4, The FT-IR spectra of cholesterol gallstones mixed with calcium carbonate consisted of the spectrum of calcium carbonate which is known to have a broad absorption band at 1474.05 cm-1. As well as sharp band at 854.35 cm-1[] in addition to the absorption bands of cholesterol at (3393.12, 2936.27, 2872.70, and 1056.70) cm-1. The asymmetric bending absorption band of the of cholesterol at 1474.05 cm-1 was superimposed on the broad absorption band of CO2- ion and the resultant band had a higher intensity than that of the pure cholesterol gallstone [5].

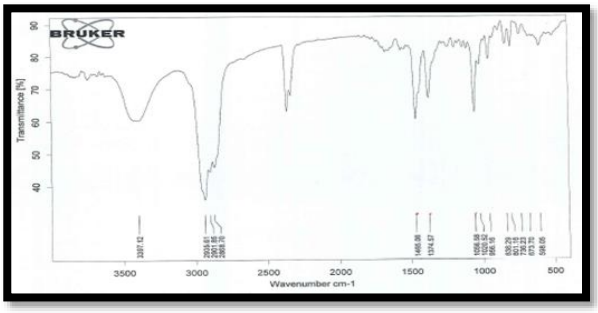

Fig. 1 FT-IR spectrum of pure cholesterol gallstone

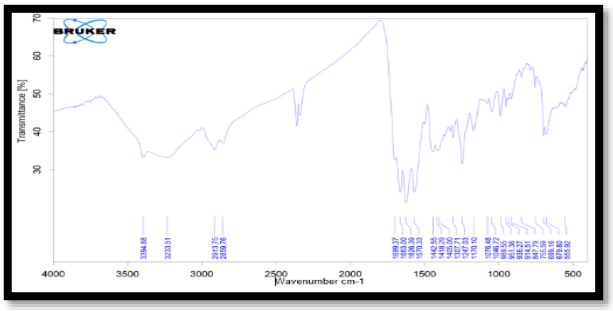

Fig. 2 FT-IR spectrum of calcium bilirubinate gallstone.

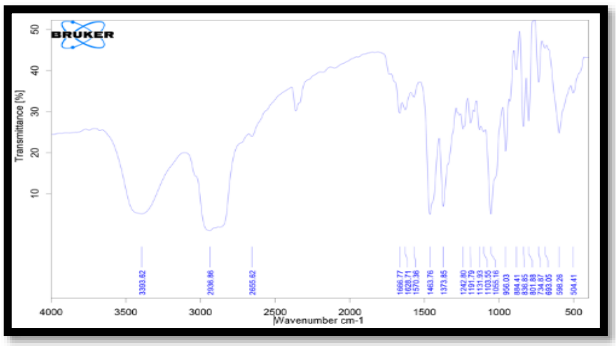

Fig.3 FT-IR specrum of cholesterol mixed with calcium bilirubinate gallstones

5-SEM and EDS techniques

The combination of SEM and EDS allows the visualization, identify the chemical characterization and mineralogical of the gallstones. Fig (5) shows the SEM of the studied gallstone samples. This figure shows plate-like cholesterol crystals with laminar
Shaped and thin layered structures. The SEM image of the mixed gallstone sample is presented in Fig. (7) With the corresponding
ED's spectrum (Fig.8). The SEM of mixed cholesterol showed different morphology from pure cholesterol stones, the stones were mainly composed of irregular plate-like cholesterol and needle-like bilirubinate deposits with white calcium salts particles. The main elements composition of mixed stones as shown by ED's spectrum

was carbon, Oxygen, calcium and few elements like Cu.

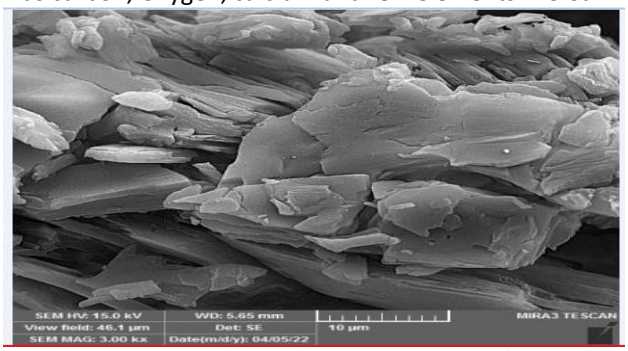

Fig. (5).scanning electron microscopy of pure cholesterol sample.

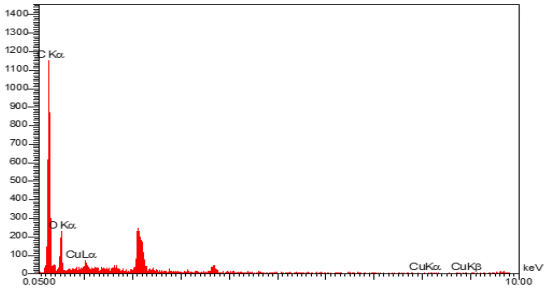

Fig. (6). EDS of pure cholesterol gallstone sample

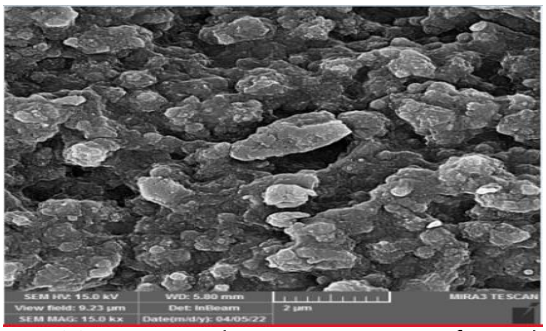

Fig (7). Scanning electron microscopy of mixed cholesterol gallstone sample

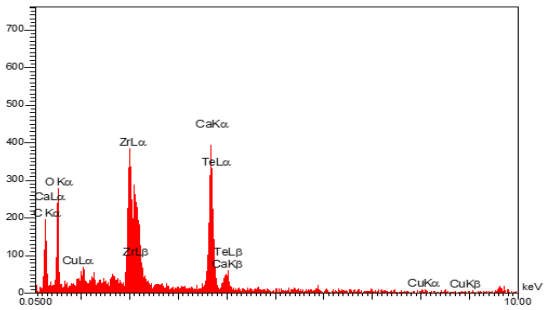

Fig. (8). EDS of mixed cholesterol gallstone sample

4. Discussion

The present study finding that majority (84.83 %) of the gallstones were pure cholesterol (30.3%) or mixed with calcium bilirubinate (46.96%) and calcium carbonate (7.57%). The important epidemiological risk factors for cholesterol gallstones included sedentary life style [9], obesity [10], female gender [11],

As compared to male (13.6%), in a ratio of female:male was (6.3:1). This is agreement with studies by other authors.some authors believes that under the influence of female sex hormones, the muscle may relax which causes to dilate biliary passage and duodenal content of pancreatic secretion regurgitates into gallbladder and promote conditions which favour the formation of gallstones. suggests that Aging [12],diabetes mellitus [13-16], femininity [11], parity [17, 18] and a diet low in calcium [19, 20], rich in saturated fats [20],and simple sugars [21]. In generally the gallstone composition mainly depends on dietary habits of the patients. The study also have been observed that gallstone disease was predominantly seen in female (86.3%)

5. Conclusion

FT-IR spectroscopy is an interested and convenient method for identification the type of gallstones, It is fast technique and required only small amount of sample. In our study the more common age group for development of gallstones was 20-49 years and female was more predominant than male. The result revealed that pure cholesterol or mixed with calcium bilirubinate or calcium carbonate is the most predominant component of gallstones in Nasiriya city, cholesterol and bilirubin are the main risk factors of gallstones diseases.

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