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Original Research Article

An analytical study of gallstones by Fourier transform infrared spectroscopy technique

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ABSTRACT

Background: Cholelithiasis continues to be a serious human health concern and presently cholecystectomy is the only adopted standard treatment option. Gallstones' chemical structures differ in different demographic groups. Accurate analysis of the constituents of gallstones may help us to understand their etiopathogenesis. Fourier transform infrared spectroscope (FTIR) can identify the composition of gallstones by creating a spectrum of infrared absorption.
Methods: A hospital based one year observational study was conducted in KLES' Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi and 120 gallstones were collected post laparoscopic/open cholecystectomy. After FTIR spectroscopic analysis gallstones were classified based on the FTIR spectrum peaks.
Results: Cholesterol, bilirubin and calcium carbonate are the components identified based on the standard FTIR spectrum characteristics with cholesterol being the commonest followed by bilirubin. Brown colored stones were more common followed by black. We classified gallstones into 3 groups based on FTIR analysis as: pure cholesterol stones, pure bilirubin stones and mixed type of stones. Mixed type of gallstones containing cholesterol and bilirubin were more common.
Conclusions: The study concludes cholelithiasis is common in females and in age group more than 40 years. Mixed types of gallstones are the commonest. Cholesterol followed by bilirubin are the major constituents of the gallstones.

Keywords: Gallstones, Gallbladder stones, FTIR analysis, Cholesterol gallstones, Cholelithiasis

INTRODUCTION

Gallstone disease continues to be a serious human health concern, affecting millions of human beings worldwide.[1] There has been a growing incidence of gallstone disease in recent years.[1] Gallstone formation is a slow process occurring over many years[2]; monitoring their formation from nucleation to consolidation is quite difficult. Therefore, gallstone formation is very poorly understood. At present surgical removal of the gall bladder is the only option available for treatment.[3]

Gallstones have traditionally been classified into four types by their gross appearance: cholesterol stones, black pigment (calcium bilirubinate) stones, brown pigment stones, and blended (mixed) cholesterol and calcium bilirubinate stones.[4] General components of all gallstones are bile and fatty acids and their calcium salts and inorganic compounds (mainly calcium carbonate).[4]

Gallstones incidence and chemical structure differ in different demographic groups.[5] Accurate analysis of the chemical composition and structure of gallstones may help us to understand their aetiopathogenesis.[6] For the determination of chemical composition of gallstones, non-spectroscopic techniques such as enzymatic and calorimetric methods have been in use. Specificity and sensitivity of these techniques are poor.[4]

Fourier transform infrared spectroscope (FTIR) can identify the composition of organic and inorganic substances by creating a spectrum of infrared absorption. The method is non-invasive, reagent-free, accurate and less time consuming.[2] The instrument is multipurpose and can therefore be used for gallstone analysis. Molecules in the gallstones provide a distinctive absorption spectrum in the infrared region, depending on the chemical bonding and the specific composition. This research is likely to enhance our knowledge of etiopathogenesis of gallstones.

The main purpose of this research is to identify the structure of various kinds of gallstones using FTIR method and to quantify their colour distinction. We also aim to classify gallstones using FTIR method and compare with the existing classification.

METHODS

Type of the study

It was a one year observational study.

The study was conducted at KAHER's Jawaharlal Nehru Medical College and KLES' Dr Prabhakar Kore Hospital and Medical Research Center, Belagavi, Karnataka, India, between January 2018 and December 2018.

Methodology

All patients undergoing laparoscopic/open cholecystectomy for gallstone disease during the study period were selected for the study. A total of 120 samples were collected by universal sampling. Gallstone samples obtained from surgically removed gallbladders after laparoscopic/open cholecystectomy after obtaining the informed consent were air-dried completely. A part of each stone was powdered and powder was then subjected to FTIR spectroscopic analysis at a frequency range of 400-4000 cm^{-1} by diffuse reflectance spectrum (DRS) method. Every sample underwent standard number of scans and the average reflectance spectrum was collected.

Gallstone classification was done based on the FTIR spectrum peak. Institutional ethical clearance was obtained for the analysis of gallstone samples.

Statistical analysis

Chi-square test or Fischer's exact test (for 2×2 tables only) was used as test of significance for qualitative data. Microsoft excel, statistical package for social sciences (SPSS) version 21 (IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

RESULTS

In the current study we have analysed 120 gallstones by FTIR spectroscopy. The absorption spectrum of each stone was compared with standard spectrum values in the literature and analysed accordingly.

Three main constituents of the gallstones have been identified in the current study; cholesterol, bilirubin and calcium carbonate based on the standard FTIR spectrum characteristics.[3,4,7]

Table 1 depicts age wise distribution of the gallstone disease.

Table 1: Distribution of age group among study subjects.

Age group (years)	Frequency	%
Less than 30	19	15.8
Between 31 to 40	25	20.8
Between 41 to 50	21	17.5
Between 51 to 60	33	27.5
More than 60	22	18.3

69.20% subjects were female and 30.80% subjects were male.

Table 2 depicts distribution of gall stones based on colour in study subjects.

Table 2: Distribution of gall stone color among study subjects.

Colour	Frequency	%
Brown	66	55.0
Black	36	30.0
Black and brown	6	5.0
Dark brown	5	4.2
Green	3	2.5
Grey	3	2.5
Red	1	0.8

Identification of components of gallstones by FTIR spectroscopy

Cholesterol

Figure 1 shows the chemical morphology of the cholesterol. Figure 2 shows FTIR spectrum characters of cholesterol in a gallstone sample.

Cholesterol molecule can be identified by the following spectrum characteristics with reference to Figure 2. Large O-H stretching peak at – 3387 cm^{-1}, C-H stretching of CH_2 peak at – 2935.66 cm^{-1}, C-H deformation peak at – 1465.9 cm^{-1}, ring deformation vibrations at – 1055.03 cm^{-1}, CH_2 symmetric stretching at – 2899.01 cm^{-1}, doublets CH_2 and CH_3 bending vibrations at – 1375.25 cm^{-1}, 1365.60 cm^{-1}, and C=C stretching vibrations at – 1664.57 cm^{-1}.

These results are comparable to a study conducted by Raman.[4]

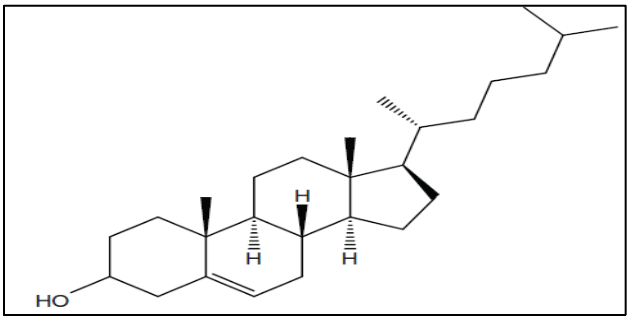

Figure 1: Chemical structure of the cholesterol.

Bilirubin

Figure 3 depicts chemical morphology of bilirubin. Figure 4 depicts FTIR spectrum characters of bilirubin in a gallstone sample.

Bilirubin can be identified based on the following characters in FTIR spectrum with reference to Figure 4. N-H stretching vibration of pyrrole groups at 3441.01 cm^{-1}, N-H stretching vibration (for lactam) at 3240 cm^{-1}, broad absorption peak of bound water at 2864.29 – 3014.74 cm^{-1}, triplet absorption peaks at 1627.92 cm^{-1} and 1658.73 cm^{-1} (unconjugated bilirubin) and 1562.34 cm^{-1} (conjugated carboxyl group in the bilirubin: calcium bilirubinate), and pyrrole ring deformation peak at 1454.33 cm^{-1}.

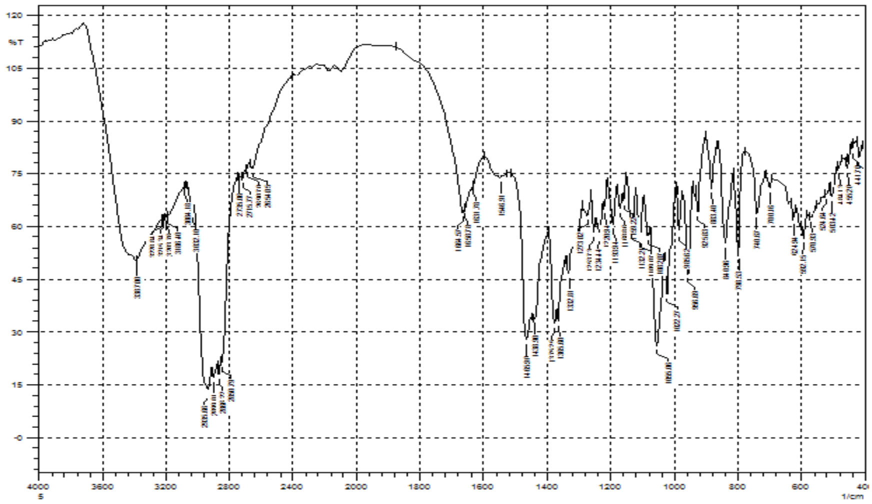

Figure 2: FTIR spectrum of gallstone (sample 5) showing characters of cholesterol.

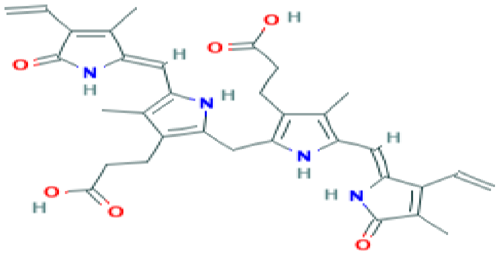

Figure 3: Chemical structure of the bilirubin.

A study conducted by Ha et al reports similar results.[8]

Calcium carbonate

Figure 5 depicts chemical morphology of calcium carbonate. Figure 6 depicts FTIR spectrum characters of calcium carbonate in a gallstone sample.

Calcium carbonate can be identified in the FTIR spectrum by the following characters with reference to the graph obtained by spectrum study of a sample (no. 74). Broad absorption peaks at 1427.32 cm^{-1} and 1480 cm^{-1}, and sharp absorption peaks at 852.54 cm^{-1} and 869.90 cm^{-1}.

A study conducted by Ha et al reports similar results.[8]

Table 3 and depicts distribution of gallstone groups.

Table 3: Distribution of gall stone groups by FTIR analysis.

Group	Frequency	%
1	11	9.2
2	9	7.5
3A	23	19.2
3B	68	56.7
3C	6	5.0
3D	3	2.5

The grouping of the gall stones is done based on the final composition analysis by FTIR technique. The group 1 consists of pure cholesterol stones, group 2 pure bilirubin stones and group 3 mixed stones. The group 3 gall stones are further classified into different subtypes based on the composition by FTIR technique. The group 3A consists of three components namely cholesterol, bilirubin and calcium carbonate. The group 3B consists of cholesterol and bilirubin. The group 3C consists of cholesterol and calcium carbonate. The group 3D consists of bilirubin and calcium carbonate.

Table 4 shows association of colour and type of gall stones.

The bilirubin type of stones presented most commonly in black (44.4%) colour followed by brown (33.3%) colour. The cholesterol type of gall stones the most common colour seen is brown (54.5%) and black (27.3%). The mixed type of gall stones presented with wide range of colours based on the composition of the stone. Brown (57%) and black (29%) is the most common colour found in mixed stones. The colour of the stones and the type of the gall stones was also found to be statistically non-significant.

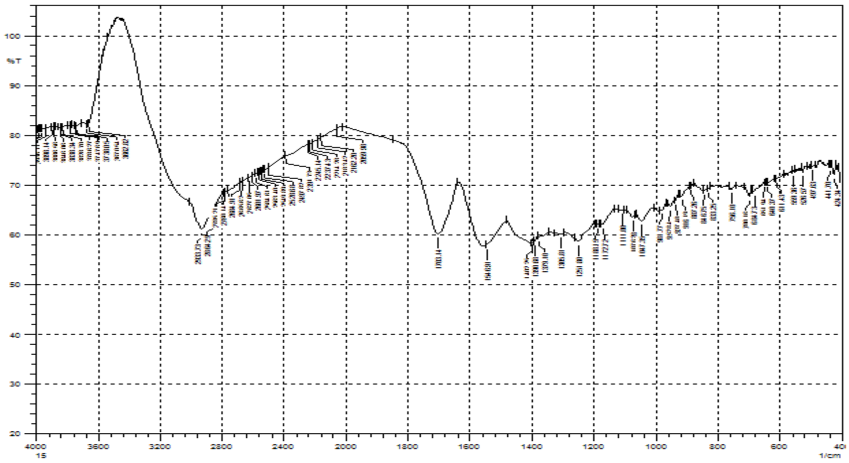

Figure 4: FTIR spectrum of a gallstone (sample 15) showing bilirubin characters.

Table 4: Association of color and type of gall stone by FTIR technique among study subjects.

Colour	Type of gallstone					
	Bilirubin		Cholesterol		Mixed	
	Frequency	%	Frequency	%	Frequency	%
Black	4	44.4	3	27.3	29	29.0
Black and brown	1	11.1	0	0.0	5	5.0
Brown	3	33.3	6	54.5	57	57.0
Dark brown	1	11.1	0	0.0	4	4.0
Green	0	0.0	1	9.1	2	2.0
Grey	0	0.0	1	9.1	2	2.0
Red	0	0.0	0	0.0	1	1.0

Chi square=8.929, and p=0.709

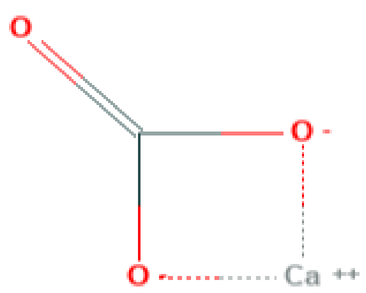

Figure 5: Chemical structure of calcium carbonate.

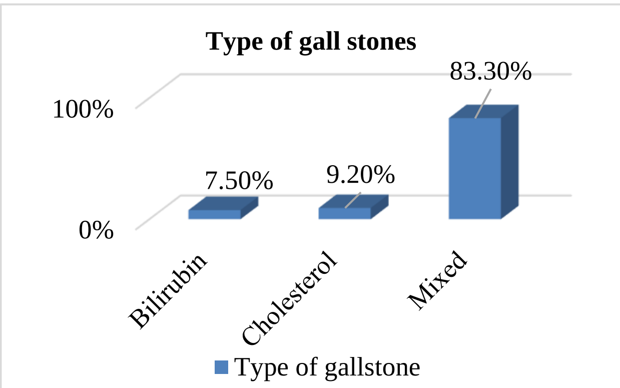

Figure 6: Graph showing distribution of the type of gall stone by FTIR analysis.

DISCUSSION

By this FTIR spectroscopic analysis we were able to classify 120 gallstones as: pure cholesterol gallstones; pure bilirubin gallstones; and mixed type of gallstones containing: cholesterol, bilirubin and calcium carbonate, cholesterol and bilirubin, cholesterol and calcium carbonate, and bilirubin and calcium carbonate.

All the absorption bands obtained in this study were comparable with those reported in the literature.[3,4,9,10] Most of the bands in this study revealed three major constituents of the gallstones: cholesterol, calcium carbonate and calcium bilirubinate. And these findings are comparable to the studies conducted by Qiao et al, Nair et al, and Lamia et al.[5,11,12]

100 out of 120 gallstones were mixed type of gallstones which was the maximum (83.3%) in this study population. The results are comparable to the studies conducted by Kleiner et al, Ravichandran et al, Raman et al and Ha et al.[2,4,8,13] Most of them were brown in colour followed by black.

Calcium carbonate has a particular absorption spectrum band which will not disturb the spectrum peaks of calcium bilirubinate or cholesterol. It can be found mixed with calcium bilirubinate, cholesterol or both. Most of these stones were brown in colour. These results are comparable with studies conducted by Ha et al and Ravichandran et al.[2,8]

Brown gallstones can be cholesterol stones or mixed stones containing all the major constituents viz cholesterol, calcium bilirubinate and calcium carbonate in various proportions. These findings are similar to the studies conducted by Shareef et al, Yoo et al, Nair et al and Cariati.[3,5,7,14]

Most of the black stones were found to be containing calcium bilirubinate, comparable with a study conducted by Venneman et al.[15] Few of these gallstones did not show absorption band peaks of other constituents namely cholesterol and calcium carbonate and accounted for 4.16% of the gallstones.

There were 3 green coloured gallstones out of which 1 stone was pure cholesterol and other two were mixed stones containing cholesterol and calcium bilirubinate. Calcium carbonate was absent in these stones.

Out of 3 grey coloured gallstones, 1 was pure cholesterol, 2 stones were mixed stones, 1 of these had cholesterol and bilirubin, and the third stone had cholesterol and calcium carbonate as constituents.

Depending upon the external appearance and colour, gallstones were conventionally classified as cholesterol stones, pigmented (black/brown) stones and mixed stones.[2] In this study based on the chemical composition and inner morphology we classified the gallstones as mentioned earlier.

This study provided us the knowledge of chemical composition and inner morphology of gallstones in a selected population in and around Belagavi which can aid in further understanding of the etiopathogenesis of the gall stone disease.

Limitations

It is possible to identify even trace elements and other compounds present in the gallstones by FTIR technique which consumes more time. FTIR spectroscopy combined with nuclear magnetic resonance studies can give precise qualitative and quantitative analysis; since it demands more time, this study was not undertaken.

CONCLUSION

We analysed 120 gallstones by Fourier Transform Infrared Spectroscopy technique and found that females are most affected by gallstone disease. It is more common after the age of 40. Mixed types of gallstones are the commonest. Cholesterol followed by bilirubin is the major constituents of the gallstones. Calcium carbonate is always seen in combination with cholesterol and/or bilirubin. Colour of the stones does not always correlate with the composition.

Recommendations

FTIR is a very versatile instrument capable of accurately analyzing variety of gaseous, liquid and solid substances. If the gall stones are analyzed completely and accurately, it may be possible to predict the precise etiopathogenesis of gall stones and devise methods of primary prevention and treatment by non-surgical methods.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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