Original article

ANALYSIS OF HUMAN GALLSTONES BY FOURIER TRANSFORM INFRARED (FTIR)

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ABSTRACT

Objective: The present study was aimed at determining the composition of gallstones removed from patients in Southern Sindh, Pakistan.

Methodology: One hundred nine (109) gallstone samples surgically removed from as many patients (98 females and 11 males; age range 20 to 80 years) admitted for treatment in Liaquat University hospital, Jamshsoro during 2000 to 2003, were analyzed for composition by Fourier Transform Infrared (FTIR) spectroscopy.

Results: Seventy four (67.9%) of the 109 gallstone samples were found to be pure cholesterol stones, Five (4.6%) pure calcium carbonate stones, Thirteen (11.9%) cholesterol + calcium carbonate, Ten (9.2%) cholesterol + bilirubin and Seven (6.4%) calcium bilirubinate stones. In mixed composition gallstones cholesterol was concentrated more at periphery than in the center of stone. Cholesterol either singly (67.9%) or in combination with either calcium carbonate (11.9%) or bilirubin (9.2%) was the most predominant component of gallstones.

Conclusion: Analysis of gallstones based on FTIR suggests that cholesterol either singly or in combination with either calcium carbonate or bilirubin is the most predominant component of gallstones in Southern Sindh, Pakistan.

KEY WORDS: Gallstones, Composition, FTIR, Cholesterol, Bilirubin, Calcium bilirubinate, Calcium carbonate, mixed gallstones.

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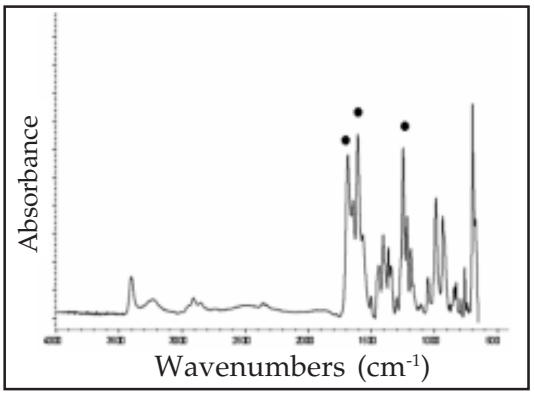
INTRODUCTION

Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world.[1,2] In Pakistan, recent years has seen an increasing trend in the number of gallstone cases in Southern Sindh, Pakistan.[3,4] 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 heterogeneous, and varies within and amongst the populations around the world.[5-10] In this article, we report the composition of 109 gallstones as obtained by using FTIR s pectroscopy.

MATERIALS AND METHODS

Patient population: There were 109 gallstone patients, 98 females (age range 20 to 80 years)

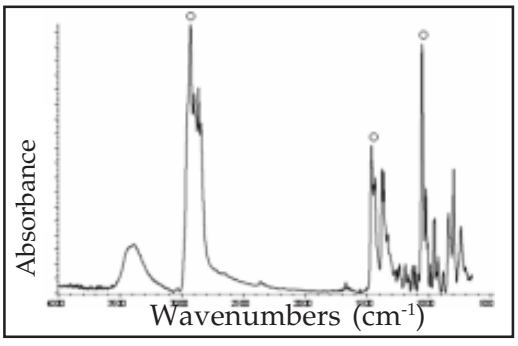


Diagnostic peaks for bilirubin

Fig-1 (a): Typical FTIR spectrum of pure bilirubin standard

and 11 males (age range 40 to 75 years). All our patients were of low socioeconomic background and resident of Southern Sindh, Pakistan. They consumed Pakistani traditional diet made of local vegetarian menu as well as pulses and very little meat mixed with locally available rice and wheat.

Stone Analysis: The materials for this study were gallstones surgically removed from 109 patients during January 2000 to December 2003. All gallstones removed during surgery were placed on sterile gauze to air dry and then washed carefully with doubly distilled deionized water (to remove bile and debris) and dried over silica gel for at least seven days. After noting the morphological features such as colour, and shape, single gallstone 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),



Diagnostic peaks for cholesterol

Fig-1 (b): Typical FTIR spectrum of pure cholesterol standard

Table-I: Percentage of cholesterol in the gallstones from 109 patients

No. of patients	*% of patients*	*Cholesterol %*
05	04.6	<25
15	13.8	25 and <50
03	02.7	50 and <80
16	14.7	80 and <100
58	53.2	= 100

and one quarter was ground with a pestle and mortar for 5 minutes. This process produced a fine homogeneous powder which was then stored in a sample tube, kept over silica gel in dark cabinet until analyzed for composition. The composition of the central part, the periphery and the stone powder was determined by using Nicolet Avatar 330 FTIR spectrometer.[11]

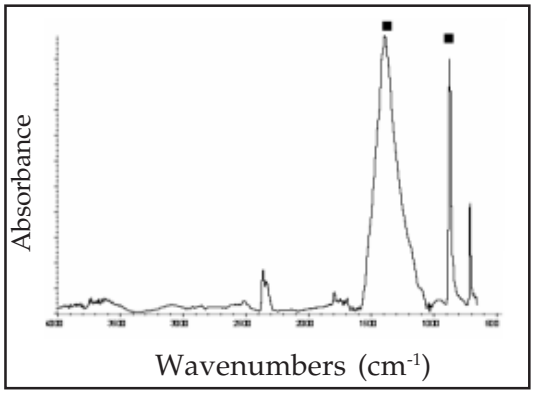
Statistical analysis: Results were computed as mean ± SD and graphs were drawn using Minitab software.

RESULTS

Figures-1 (a), (b) and (c) show the typical FTIR spectra of pure bilirubin, cholesterol, and calcium carbonate standards respectively.

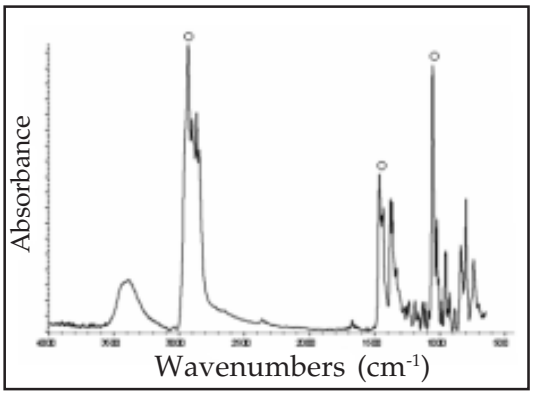
Table-I shows the breakdown of gallstones according to percentage of cholesterol content. As can be seen, majority of the gallstones (53.2%) had 100% cholesterol content.

Table-II presents the types of gallstones identified and their frequency of occurrence along with the principal IR bands for each component. Of the 109 gallstones analyzed, 74 (67.9%) were identified as pure cholesterol



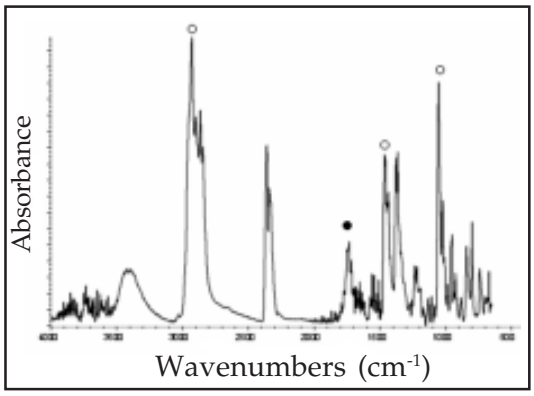
Diagnostic peaks for calcium carbonate

Fig-1 (c): Typical FTIR spectrum of pure $CaCo_3$ standard.



Cholesterol

Fig-2: Typical FTIR spectrum of cholesterol gallstone sample.



Cholesterol; bilirubin

Fig-3: Typical FTIR spectrum of cholestero + bilirubin gallstone sample.

stones, 5 (4.6%) and 7 (6.4%) as pure calcium carbonate and calcium bilirubinate gallstones respectively, and the remaining 23 (21.1%) were mixed component gallstones. Of the mixed component gallstones 13 (11.9%) were mixtures of cholesterol + calcium carbonate and 10 (2%) of cholesterol + bilirubin. The diagnostic bands identified for cholesterol (Figure 2) were the strong bands around 2929, 1463 and 1054 cm^{-1}. Similarly the diagnostic bands for bilirubin (Figure-3); for calcium bilirubinate (Figure-4); and for calcium carbonate (Figure-5) were 1683, 1607 and 1246; 390,1660 and 1435; 1028 and 854 cm^{-1} respectively. Comparison of composition of different parts of gallstones (central part, the periphery and the whole stone) is shown in figure-6. It can be seen that the highest concentration for cholesterol and bilirubin occurred at periphery. Cholesterol was present in highest concentration in all parts of the gallstones.

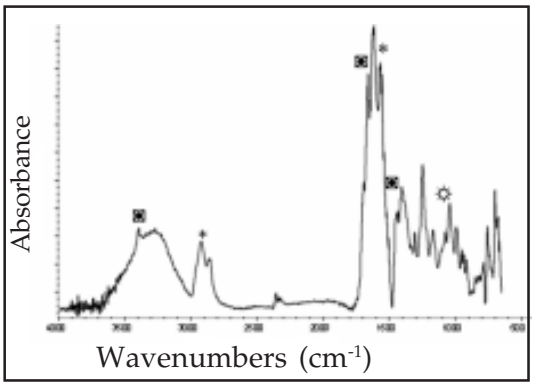
DISCUSSION

The gallstones which contained more than 80% cholesterol were classified as pure cholesterol gallstones.[12] Pure cholesterol gallstones were characterized by the bands around 2929, 1463 and 1054 cm^{-1}. Cholesterol in the 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.[13]

The characteristic band features and key band locations for the components of gallstones in the present study were in accordance with those reported in the literature.[11,14] Pure cholesterol gallstones were much more common than the gallstones containing cholesterol admixed with protein, bilirubin, and carbonate (Figures 4, 5, and 6). Black pigment stones were dark brown or black, small, multiple, and had an irregular surface; they were composed

Table-II: Type, occurrence, and IR bands of principal components observed in gallstones

Type of gallstone	*Frequency of occurrence of stones*	*Principal IR bands observed in present study*	*Literature values (Reference [Zhou X-S 1997])*
Pure cholesterol	74	2929, 2899, 2865, 1463, 1054	2925 (CH_2 and CH_3 asymmetric stretching), 2860 (CH_2 and CH_3 symmetric stretching) 1460, (CH_2 and CH_3 bending), 1050 (C-C stretching)
Pure calcium carbonate	05	1338, 854	1481, 855 [O Kliener]
Cholesterol + calcium carbonate	13	Same as above and 1338, 854	Same as above and 1481, 855 [O Kliener]
Cholesterol + bilirubin	10	Same as in pure cholesterol and 1683, 1607, 1246	Same as in pure cholesterol and 1670, 1640 (OC=O stretching), 1575 (C=C stretching)
Calcium bilirubinate	07	3390, 1660, 1435	3410 (CH_2 and CH_3 asymmetric stretching), 1380 (CH_2 and CH_3 bending)



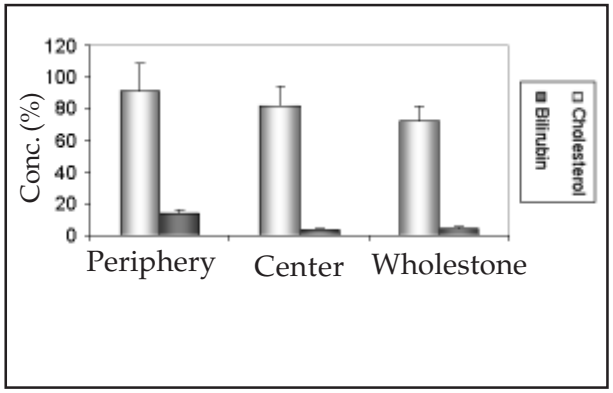
Calcium bilirubin; cholesterol; fatty acids; calcium carbonate; protein; bilirubin

Fig-4: Typical FTIR spectrum of calcium bilirubinte gallstone sample.

of calcium bilirubinate along with large amounts of fatty acids and almost no calcium carbonate. The prevalence of calcium bilirubinate stones in the present series was 6.4%. It was <1% in Bolivia, 5% in Texas, 9% in Japan, 30% in the eastern United States, and 40% in India.[15]

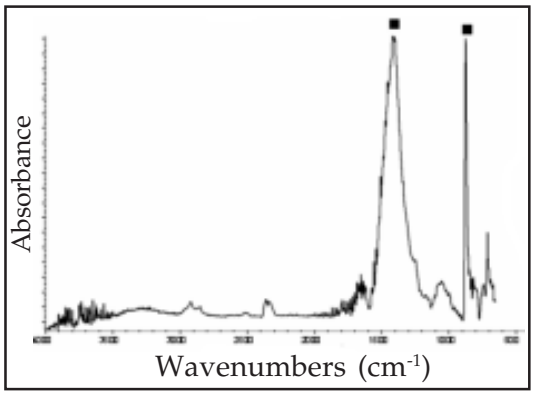
As far as pure cholesterol gallstones were concerned, some had a radial structure from the center to the periphery on cross section,[16] whereas most had pigment at the center and grossly visible cholesterol crystals at periphery.[16,17] The chemical composition of pigmented centers of cholesterol gallstones was quantitatively different from that of black pigment stones, suggesting that cholesterol gallstones do not form on a pigment stone nidus.[17]

The present finding that majority (67.9%) of the gallstones were pure cholesterol stones and



Calcium carbonate

Fig-6: Composition of different parts of gallstones as analyzed by FTIR



Calcium carbonate

Fig-5: Typical FTIR spectrum of pure calcium carbonate gallstone sample.

that the concentration of cholesterol was markedly high, both in the periphery and center of the gallstones confirms the reports of previous workers from Pakistan that pure cholesterol gallstones are far more prevalent than the pigment and mixed component gallstones.[3,18,19] Cholesterol gallstone disease is indeed a multifactorial disease. The important epidemiological risk factors for cholesterol gallstone formation include sedentary life style,[20] obesity,[21,22] diabetes mellitus,[22–25] aging,[26] female gender,[27] psychological stress,[28] femininity,[27] parity,[29,30] and a diet low in calcium,[31] rich in saturated fats[32,33] and simple sugars.[31,34] Additional dietary factors associated with cholesterol gallstone disease are consumption of rapeseed oil, cottonseed oil, butter,[35] legumes,[36,37] beans,[38-40] raw green chilies, saag, palak, tomatoes and tea.[41-43] Although, it is generally agreed 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 gallstones.[44]

As such, we believe that more thorough investigations are required to clarify the role of specific dietary components in the pathogenesis of cholesterol gallstones in this area.

CONCLUSIONS

Our results suggest that cholesterol either singly or in combination with calcium carbonate or bilirubin is the most predominant component of human gallstones in Southern Sindh, Pakistan.

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