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UTILIZE THE FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY TO ANALYZE THE CHEMICAL COMPOSITION AND STRUCTURAL CHARACTERISTICS OF GALLSTONES

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ABSTRACT

Fourier Transform Infrared (FTIR) Spectroscopy is an advanced analytical technique used to elucidate the chemical composition and structure of materials by measuring their infrared absorption spectra. This study explores the application of FTIR spectroscopy in the analytical study of gallstones, a common condition with significant clinical implications. Gallstones, formed primarily of cholesterol, bilirubin, and calcium salts, vary in composition and can lead to diverse health issues such as cholecystitis and pancreatitis. This study underscores the utility of FTIR spectroscopy as a non-destructive, rapid, and cost-effective method for analyzing gallstone composition. Its application can aid in clinical diagnostics by providing insights into the type and cause of gallstone formation, ultimately contributing to improved management and treatment strategies. Future advancements in FTIR technology and its integration with other analytical methods are expected to further enhance its role in both research and clinical settings.

Aim: The primary aim of this study is to utilize Fourier Transform Infrared (FTIR) Spectroscopy to analyze the chemical composition and structural characteristics of gallstones.

Material and method: This observational study was conducted in the department of General surgery. 50 gallstones, collected from gallbladders after cholecystectomy, were provided by the department of internal medicine without any patient information. All patients undergoing laparoscopic/open cholecystectomy for gallstone disease during the study period were selected for the study. A total of 50 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.

Results: The absorption spectrum of each stone was compared with standard spectrum values. 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. 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.

Conclusion: FTIR spectroscopy is a valuable tool for the analytical study of gallstones, providing detailed information on their composition and aiding in both clinical and research contexts. 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. By addressing its limitations and leveraging advancements in technology, FTIR can continue to contribute significantly to the understanding and management of gallstone-related conditions.

Keywords: Gallstones, Gallbladder stones, FTIR analysis, Cholesterol gallstones, Cholelithiasis, Pigment Stones, Mixed Stones.

INTRODUCTION

Gallstones are crystalline deposits that form within the gallbladder, an organ responsible for storing bile produced by the liver. Gallstones, also known as cheilitis, are solid particles that form in the gallbladder and can lead to various clinical conditions, including cholecystitis, pancreatitis, and biliary obstruction. They can vary in size, shape, and chemical composition, typically categorized into three main types: Cholesterol Stones: Predominantly composed of cholesterol. These stones are often yellow and can range from small granules to large stones. Pigment Stones: Composed mainly of bilirubin and calcium salts. These stones are usually smaller, darker, and can be either black or brown. Mixed Stones: Contain a combination of cholesterol, bilirubin, and calcium salts, presenting a mixed composition.[1]

FTIR spectroscopy is a versatile analytical technique used to identify molecular components and functional groups within a sample by measuring the absorption of infrared light at different wavelengths. This technique relies on the principle that different chemical bonds absorb infrared radiation at specific frequencies, leading to a unique spectral "fingerprint" for each substance. [2] Infrared Radiation Absorption: Molecules absorb infrared radiation at specific wavelengths, causing vibrational transitions in the bonds. This absorption creates a spectrum with peaks corresponding to these vibrational frequencies. Fourier Transform: FTIR utilizes a Fourier Transform to convert raw data from an interferogram into a detailed spectrum. This transformation allows for high-resolution and rapid spectral acquisition.[3]

FTIR spectroscopy is based on the principle that molecules absorb infrared radiation at specific frequencies corresponding to the vibrational modes of their chemical bonds. When infrared light passes through a sample, certain wavelengths are absorbed, causing changes in the vibrational energy of the molecules. The resulting absorption spectrum provides a "fingerprint" that can be used to identify and quantify the components of the sample.

Fourier Transform Infrared (FTIR) Spectroscopy is a powerful analytical technique used to identify and characterize materials based on their infrared absorption spectra. When applied to the study of gallstones, FTIR can provide valuable insights into their chemical composition and structure. FTIR is a non-destructive technique, meaning samples can be analyzed without altering or damaging them. The technique often requires minimal sample preparation, making it convenient for analyzing various types of samples. [4]

FTIR provides both qualitative information about the chemical composition with appropriate calibration and quantitative data on the concentration of different components. FTIR can precisely identify the different chemical components of gallstones by detecting specific functional groups associated with cholesterol, bilirubin, and calcium salts. By analyzing the FTIR spectra, researchers can classify gallstones into cholesterol, pigment, or mixed types based on their spectral profiles. Understanding the chemical composition of gallstones can provide insights into the metabolic or pathological conditions leading to their formation, such as hypercholesterolemia or bilirubin disorders.

FTIR spectroscopy can be used in both research settings to study the formation mechanisms of gallstones and in clinical diagnostics to guide treatment decisions. [5]

FTIR spectroscopy is a robust and versatile technique that offers detailed insights into the molecular composition and structure of a wide range of materials. Its ability to provide both qualitative and quantitative information makes it an invaluable tool in many scientific and industrial fields. FTIR spectroscopy is a valuable tool for the analytical study of gallstones, offering detailed insights into their chemical composition and type. The technique's ability to provide non-destructive, high-resolution molecular data makes it an essential method in both clinical diagnostics and research. Despite some limitations, such as water interference and complex spectra, ongoing advancements continue to enhance its application and accuracy in gallstone analysis.

Material and methods

This observational study was conducted in the department of General surgery. 50 gallstones, collected from gallbladders after cholecystectomy, were provided by the department of internal medicine without any patient information. All patients undergoing laparoscopic/open cholecystectomy for gallstone disease during the study period were selected for the study. A total of 50 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. All our patients were of low socioeconomic background and resident of India. They consumed Indian traditional diet made of local vegetarian menu as well as pulses and very little meat mixed with locally available rice and wheat.

FTIR Spectrometer:

- A Fourier Transform Infrared (FTIR) spectrometer equipped with necessary accessories for sample analysis.
- Key components include an infrared light source, interferometer, detector, and computer system for data processing.

Sample Preparation Materials:

- **Potassium Bromide (KBr)**: For preparing KBr pellets if analyzing solid samples.
- **Sodium Chloride (NaCl) Windows**: For liquid samples, if applicable.
- **Grinding Equipment**: Mortar and pestle or mechanical grinder to pulverize solid samples.
- **Pressing Device**: Pellet press for creating KBr pellets.

Additional Reagents and Equipment:

- **Cleaning Agents**: For cleaning sample holders and accessories.
- **Desiccant**: To ensure samples are dry, if necessary.
- **Protective Gear**: Lab coat, gloves, and safety goggles for handling samples and chemicals

Sample Preparation

For Solid Gallstones:

- **Cleaning**: If necessary, clean the gallstones to remove any surface contaminants. This may involve washing with distilled water and drying.
- **Grinding**: Crush the gallstones into a fine powder using a mortar and pestle or mechanical grinder. Ensure the sample is uniformly powdered.

KBr Pellet Preparation:

- Mix the powdered gallstone sample with KBr (typically at a 1:100 ratio) to create a homogeneous mixture.
- Press the mixture into a thin, translucent pellet using a pellet press. The KBr serves as a matrix that is transparent to infrared radiation, allowing the analysis of the gallstone sample.

Sample Analysis:

- **Sample Placement**: Place the prepared sample (KBr pellet or liquid) in the sample compartment of the FTIR spectrometer.
- **Data Collection**: Record the FTIR spectrum over a range of wavelengths (typically 4000 cm^{-1} to 400 cm^{-1}) to capture the infrared absorption features of the sample. Every sample underwent standard number of scans and the average reflectance spectrum was collected. photographs of gallstones from

each group were collected and compared with each other to identify the presence of a common morphology in the same group.

Statistical Analyses

Chi-square test or Fischer's exact test was used as test of significance for qualitative data. Microsoft excel, statistical package for social sciences (SPSS) version 21 (was used to analyse data.

Result:-

In the current study we have analysed 50 gallstones by FTIR spectroscopy. The absorption spectrum of each stone was compared with standard spectrum values. 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.[3,4,6]

Table 1: shows the Distribution of gall stone color among study subjects

Colour	Frequency	Percentage (%)
Brown	16	32
Black	21	42
Blackish brown	6	12
Dark brown	4	8
Green	1	2
Grey	1	2
Red	1	2

Table 2: shows the Distribution of gall stone groups by FTIR analysis

Group	Frequency	%
1	10	20
2	5	10
3A	11	22
3B	15	30
3C	6	12
3D	3	6

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 3: shows the 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	3	6	2	4	11	22
Black and brown	1	2	0	0	3	6
Brown	2	4	4	8	15	30
Dark brown	1	2	0	0	3	6
Green	0	0	1	2	2	4
Grey	0	0	1	2	2	4
Red	0	0	0	0	2	4

The bilirubin type of stones most commonly presented in black (6%) colour followed by brown (4%) colour. The cholesterol type of gall stones the most common colour seen is brown (8%) and black (30%). The mixed type of gall stones presented with wide range of colours based on the composition of the stone. Brown (30%) and black (22%) 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.

Discussion:

Fourier Transform Infrared (FTIR) Spectroscopy provides a detailed molecular profile of gallstones, offering valuable insights into their chemical composition and formation. FTIR spectra of gallstones reveal specific absorption bands that correspond to different functional groups, allowing for the identification and characterization of the stone's composition: **Cholesterol Stones:** FTIR spectra typically show prominent peaks related to cholesterol's hydroxyl (OH) stretching at approximately 3300 cm^{-1}, CH stretching at around 2920 cm^{-1}, and C=O stretching at around 1730 cm^{-1}. The clear identification of these peaks helps confirm the presence of cholesterol as the primary component. **Pigment Stones:** These stones exhibit characteristic peaks associated with bilirubin and calcium salts. Absorption bands around 1700 cm^{-1} correspond to the C=O stretching in bilirubin, while peaks around 1400 cm^{-1} are indicative of carbonate groups in calcium salts. The presence of these peaks confirms the composition and type of pigment stones. **Mixed Stones:** Mixed stones show a combination of peaks from both cholesterol and pigment stones, reflecting their diverse composition. The overlap of peaks necessitates advanced spectral analysis techniques to deconvolute and accurately identify the components.[7,8]

Accurate characterization of gallstone composition can assist in diagnosing underlying metabolic disorders and guide treatment strategies. For example, identifying cholesterol stones may indicate hypercholesterolemia, while pigment stones could suggest liver dysfunction or hemolytic disorders.

FTIR spectroscopy contributes to research on gallstone formation mechanisms and potential preventive measures. By understanding the chemical composition and structural variations, researchers can explore new therapeutic options and develop better diagnostic tools. Insights gained from FTIR analysis can support personalized medicine approaches by tailoring treatment plans based on the specific type and composition of gallstones in individual patients. Continued improvements in FTIR instrumentation, such as better detectors and higher-resolution optics, will provide more detailed spectral information and enhance the detection of minor components. Combining FTIR with complementary techniques, such as mass spectrometry or X-ray diffraction, could offer a more comprehensive understanding of gallstone composition and structure. Portable and handheld FTIR devices could facilitate in-situ analysis of gallstones, making the technique more accessible in clinical and field settings.[9,10]

Byeong Jo Ha et al. found FT-IR spectra of nine gallstones matched well with that of pure cholesterol, and the gallstones were thus classified as cholesterol stones. Twelve gallstones were classified as calcium bilirubinate stones as they showed characteristic absorption bands of calcium bilirubinate. However, the FT-IR spectra of these gallstones always showed a broad absorption band of bound water at 3600–2400 cm^{-1}. The other five gallstones were classified as mixed stones with combinations of cholesterol, calcium bilirubinate, and calcium carbonate.[9]

Brown gallstones should be classified with care, as they could be a cholesterol gallstone covered with a very thin brown layer, a mixed gallstone of cholesterol with calcium bilirubinate and calcium carbonate, or a mixed gallstone of calcium bilirubinate with calcium carbonate. 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.

A side-by-side comparison of the FT-IR spectrum of a gallstone with its corresponding photographic image was attempted previously for selected gallstone samples, and it helped to understand the chemical composition and morphology of that gallstones.[10]

Conclusion:

FTIR spectroscopy is a valuable tool for the analytical study of gallstones, providing detailed information on their composition and aiding in both clinical and research contexts. 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. By addressing its limitations and leveraging advancements in technology, FTIR can continue to contribute significantly to the understanding and management of gallstone-related conditions.

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