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Compositional study of laproscopically removed gallstones

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Abstract

The Gallstone disease has become one of the major surgically related issues among the people; it is most likely associated with nutrition, particularly overeating meat. The formation of gallstones is comparatively high with the intake of nutrients that are rich in saturated fatty acids. Recently, the number of cases in Pakistan has been rising with each passing day. This present study has focused mostly on determining the gallstone composition removed by laparoscopic stone surgery from patients admitted to the department of surgery of Civil Hospital of Ghulam Mohammad Mahar Medical College Hospital Sukkur, Sindh, Pakistan. The analysis of the composition of the patient's gallstones is performed by the FTIR during 2018 to 2019. Fifty (50) stones specimens removed by laparoscopic stone surgery from patients were gathered and examined. Out of them eight (08) specimens were of males and forty two (42) of females. And 4% (2/50) of specimens were found to be calcium bilirubinate stones, 6% (3/50) of pure calcium carbonate, 12% (6/50) of cholesterol + calcium carbonate, 12% (6/50) of cholesterol + bilirubin and 66% (33/50) of cholesterol stones. Investigation of gallstones using FTIR Spectroscopy showed that cholesterol was the dominant constituent in gallstones.

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Introduction

Gallstones, complex biomineralized deposits formed in the gallbladder (Kravets & Moskalenko, 2016; Wentrup-Byrne *et al.,* 1997), is yet a main health issue worldwide (Sharma *et al.,* 2016). 20% to 30% population of western and about 10% population of non-western are suffering from gallstones disease (Harding, 1964; Kern, 1983). Recently, the number of cases in Pakistan has been rising with each passing day (Channa *et al.,* 2008). The Gallstone disease has become one of the major surgically related issues among the people; it is most likely associated with nutrition, particularly overeating meat. The formation of gallstones is comparatively high with the intake of nutrients that are rich in saturated fatty acids (Jaraari *et al.,* 2010; Tsai *et al.,* 2004). It is unfortunate that the composition of Gallstone is heterogeneous and variable both within and between communities worldwide (Kalloo & Kantsevoy, 2001; Kleiner *et al.,* 2002; Kratzer *et al.,* 1999). The main elements implicated in the creation of stones in the gallblader of humans are calcium, biliary pigment and cholesterol (Moosavi *et al.,* 2006). Regarding chemical composition, most of the stones in human are pigment and cholesterol (Sahuquillo *et al.,* 2000; Singh *et al.,* 2008). Gallstones of cholesterol are composed of over 70% cholesterol and even a minor content of trace elements and pigment residues (Fauziah *et al.,* 2016; Iqbal *et al.,* 2019; Ravnborg *et al.,* 1990). Pigment stones contain calcium bilirubinate as the main component and can further be divided into black and brown stones (Vítek & Carey, 2012).

Stones in gallbladders may cause symptoms or they may remain asymptomatic. More than half of the cases have no symptoms, often seen on ultrasound of the abdomen (Roslyn *et al.,* 1987). Epidemiology study states that 70-80% of gallstone disease remains asymptomatic and approximately 20% cause symptoms with complications that emerge in 5-20% years after diagnosis. Gallstone passes through gallbladder to the intestine is potential to cause ileus and require surgical procedure (Acalovschi, 2014; Fitzgerald *et al.,* 2009). For the determination of gallstones' chemical composition, non-spectroscopic techniques, like calorimetric and enzymatic techniques, have been used. These methods have a low degree of specification, sensitivity and imprecise values. IR spectroscopy is the extensively employed technique due to its advantages over chemical analysis or any other technique.

It requires a very low volume of sampling, the specificity of each constituent and gives quantitative outcomes with higher reproducibility (Yoo *et al.,* 2008). FT-IR spectroscopic method is a valid technique for evaluating the composition of gallstones (Ishida *et al.,* 1987). Therefore in this current research work we analyzed the composition of gallstones by utilizing the FTIR spectroscopy.

Materials and methods

Patient population

All Fifty (50) stone specimens removed by laparoscopic stone surgery from patients were gathered and examined. Out of them 08 specimens were of males and 42 of females. Their ages ranges from 15 to 63 years and their male to female ratio was 1:5.25 as shown in Table 1 and Fig. 1. The analysis of the composition of the patient's gallstones is performed by the FTIR spectroscopy.

Analysis of Stone specimens

The gallstones, materials being studied, have been gathered from the infected persons by laparoscopic surgery. During 2018-2019, fifty subjects were assessed. The collected specimens have been dried using sterile gauze, bile and residues have been disposed of with de-ionized H_2O, and they have been left on silica gel to dry for one week. The stones were cut to four equally-sized parts and 1/4 of each was ground with a pestle and mortar entirely and later brought to a test tube and mixed with different solvents for their dissolution based on the nature of their components and finally examined using the FTIR spectroscopy.

Results and discussion

It was observed in current study that the females 84% (42/50) are at higher risk of formation of gallstones disease than males 16% (08/50), their age ranges from 15 to 63 years and their male to female ratio was 1:5.25 as shown in Table 1 and Fig. 1.

The highest incidence of gallstone disease was observed in the age group 35-55 in 25/50 (50%) (05 males + 20 females) patients followed by 10/50 (20%) (01 male + 09 females) in the age group 15-34 and 15/50 (30%) (02 males + 13 females) in the age group 56-63 as shown in Table 1 and Fig. 2.

Table 1. Gender-wise distribution of patients and Age-wise prevalence of the gallstone disease.

Gender-wise distribution of patients		
Gender	Number of patients (%)	
Male	08 (16%)	
Female	42 (84%)	
Male: Female Ratio	1:5.25	
Age-wise prevalence of the gallstone disease		
Age group	Sex	Percentage%
15- 34	01 male + 09 female = 10	(20%)
35 – 55	05 male + 20 female = 25	(50%)
56 – 63	02 male + 13 female = 15	(30%)
Total	08 male + 42 female = 50	(100%)

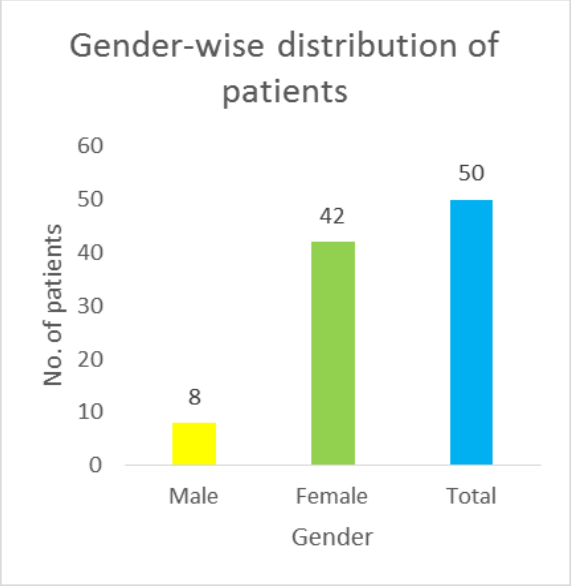

Fig. 1. Gender-wise distribution of patients.

In the present work, the FTIR has been employed as a fundamental tool in the assessment of gallstones chemical composition, as it is a more accurate analytical technique. Among the main benefits of the FTIR in the analysis of gallstones is the capability to utilize just a minimum quantity of specimen for tests. For the identification of the compounds chemically, the wavenumbers got for the specimens were compared with the standards of cholesterol, pure bilirubin and $CaCO_3$. The closest possible match has been assured to reduce interpretation errors.

Fig. 2. Age-wise prevalence of the gallstone disease.

In addition, the wave numbers obtained have been matched with data from literature available for improvement in accuracy. Bands for Cholesterol specimens were observed at 3439 cm^{-1} due to the large CH_2 asymmetric stretching vibration, CH_3 asymmetric stretching at 2935.14 cm^{-1}, CH_2 bending at 1470.21 cm^{-1} and a sharp peak at 1060cm^{-1} can be attributable to ring deformation of cholesterol. FT-IR spectra of a cholesterol stones in combination with bilirubin comprising cholesterol characteristic bands for absorption at 3439, 2935.14 and 1060cm^{-1}. Though, the absorption peaks of CH_2 asymmetric bending for cholesterol at 1470.21cm^{-1}. Moreover, the characteristics absorption peaks of bilirubin were at 1681.97cm^{-1} for OC=O stretching band and 1569.82cm^{-1} for C=C stretching. Band for Calcium bilirubinate specimens were observed at 3396.37cm^{-1} due to the large CH_2 and CH_3 asymmetric stretching vibration, OC=O stretching band at 1630.10cm^{-1} and CH_2 bending at 1470 cm^{-1}. FT-IR spectra $CaCO_3$ is recognized by having a wide peaks of absorption at 1420cm^{-1} to 1480 cm^{-1}, and also sharp peaks of absorption at 855cm^{-1} and 872cm^{-1}. In this study a broad absorption peaks and sharp absorption peaks for pure calcium carbonate were at 1450.17cm^{-1} and 865cm^{-1} respectively. FT-IR spectra of a cholesterol stones in combination with $CaCO_3$ comprising cholesterol characteristic bands for absorption at 3439, 2935.14 and 1060cm^{-1}.

Though, the absorption peaks of CH_2 asymmetric bending for cholesterol at 1470.21cm^{-1} was overlaid on wide band absorption of carbonate ions so resulting peak ensured a greater intensity as compared to gallstone of pure cholesterol. Absorption peaks of symmetric bending of CH_2 for was at 1470.21cm^{-1} seemed like a shoulder band for absorption of the carbonate at 1449.89cm^{-1}. Moreover, the characteristics absorption peaks of CO_3^{-2} was detected at 863.42cm^{-1} for gallstones of cholesterol in combination with $CaCO_3$ as shown in Table 2. The bands for the gallstones components were according to the literature reported as shown in Table 2.

Table 2. Occurrence, percentage, type and principal FTIR bands of constituents of gallstones.

Frequency of specimens	Types of gallstones	% age	Principal FTIR bands examined	Assingments (Ha *et al.,* 2018; Kliener *et al.,* 2002; Zhou *et al.,* 1997)
33	Pure Cholesterol	66%	3439	CH_2 asymmetric stretching
			2935.14	CH_3 asymmetric stretching
			1470.21	CH_2 bending
			1060	ring deformation of cholesterol
06	Cholesterol + bilirubin	12%	Similar bands as of pure cholesterol	Similar assignments as of pure cholesterol
			1681.97	OC=O stretching
			1569.82	C=C stretching
02	Calcium bilirubinate	04%	3396.37	CH_2 & CH_3 asymmetric stretching
			1630.10	OC=O stretching
03	Pure calcium carbonate	06%	1470	CH_2 bending
			1450.17	carbonate absorption band (1420-1480cm^{-1})
			865	sharp absorption peaks of carbonate (872 and 855cm^{-1})
06	Cholesterol + calcium carbonate	12%	3439	
			2935.14	Similar assignments as of pure cholesterol
			1470.21	
			1060	
			1449.89	carbonate absorption band
			863.42	sharp absorption peaks of carbonate

4% (2/50) of specimens were found to be calcium bilirubinate stones, 6% (3/50) of pure calcium carbonate, 12% (6/50) of cholesterol + calcium carbonate, 12% (6/50) of cholesterol + bilirubin and 66% (33/50) of cholesterol stones as shown in Table 2. This finding demonstrates that pure cholesterol gallstones were proportionally higher (66%) and also verifies the work already undertaken over it in Pakistan that gallstones of pure cholesterol are common compared to others (Channa *et al.,* 2008; Khand, 1997). The Gallstone disease has become one of the major surgically related issues among the people; it is most likely associated with nutrition, particularly overeating meat. The formation of gallstones is comparatively high with the intake of nutrients that are rich in saturated fatty acids (Jaraari *et al.,* 2010; Tsai *et al.,* 2004). Previous researches have reported that gallstone formation risk was pleasantly related to meat, fat and saturated fat intake, whereas it was adversely related to plant and fibre intake in Asian and Western countries. Particularly, intakes of lot of flesh were found to be related to the risk of gallstone disorders, as eating of red meats suppressed the bile acid carriers of trimethylamine that were responsible for the induction of cholesterol gallstones (Park *et al.,* 2017).

Conclusion

It is concluded from the findings of the present investigation that the most common gallstone removed by laparoscopic stone surgery from patients admitted to the department of surgery of Civil Hospital of Ghulam Mohammad Mahar Medical College Hospital Sukkur, Sindh, Pakistan was cholesterol stone and females suffered more from gallstone disease than males, with male to female ratio of 1:5.25.

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