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# Estimation of stones in gallbladder with FT-IR

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**Key words:** Fourier transform infrared spectroscopy, Calcium bilirubinate, Cholesterol gallstones

## Abstract

Forty (40) specimens of the gallstones were taken from the infected peoples who were admitted and cured in the Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, Nawabshah, Sindh, Pakistan. In our work gallstones (total 40) were found in the age ranges from 20-60 years for male (07) patients and from 20-65 years for female (33) patients. The greatest ratio of gallstones was found 21/40 in the age ranges 36-50 years of affected peoples. The occurrence of gallstones was higher in females than males and sex ratio was found to be 1:4.7 male to female. The size and weight of gallstones vary and the size and weight of pure cholesterol stones were calculated as 0.36-2.27cm and 0.231-0.964g respectively while calcium carbonate stones were as 0.8-2.1cm and 0.305-0.646g respectively and 1.4-2.2cm size and 0.307-0.853g weight for calcium billirubinate gallstones were measured. In current work the cholesterol gallstone was the most common type of gallstones. The 28 (70%) of sample stones were detected as pure cholesterol gallstones while pure calcium carbonate were 05 (12.5%) and 07 (17.5%) were calcium bilirubinate out of 40 specimens of gallstones. The 28 gallstones were irregular and 12 were round in shape. Moreover, From 40 gallstone specimens 29 were found with smooth surfaces while 11 were with rough surfaces.

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## Introduction

Medically, the process of forming a gallstone, is called cholelithiasis (Zhou *et al.,* 1997) which is usually a slow or long-term process that generally does not cause pain or other symptoms (Gurusamy and Davidson, 2014; Reshetnyak, 2012). Gallstones grow when some biliary solutes, such as cholesterol or calcium chloride, are precipitated as solid crystals and gradually grow into a gallbladder mucous lining (Maurer *et al.,* 2009). Gallbladder is a slender pear-shaped organ in a right superior belly (Le Bail *et al.,* 1992), the region below the liver which links chest to hips. Gallstones can vary between a1cm and 13cm in size. Single or multiple gallstones have various shapes, e.g. oval or circular, irregular or smooth surfaces (Bassi *et al.,* 1994). Gallstones typically are white, yellow, brown, black, and green (Sikkandar *et al.,* 2011). Gallstone components must be identified because it offers details that could be helpful to physicians in determining the root cause of gallstone and deciding whether patients with gallstones should be treated therapeutically or surgically (Channa *et al.,* 2007). Unfortunately, the composition of gallstones is complex, and varies throughout and across the population of the world (Kratzer *et al.,* 1999; Kleiner *et al.,* 2002; Kalloo & Kantsevoy, 2001). Pure cholesterol stone prominence is much more common in Pakistan than pigment and mixed calculi.

Gallstone disease is a major public health issue, associated with hospital admissions and surgical interventions. It is one of the most prevalent and costly gastrointestinal diseases, resulting in the Asian population having a large economic burden ranging from 3 to 15 per cent. Gallstone occurrence is most commonly associated with the dietary factors. The research study carried out on the composition of gallstones in various locations around the world demonstrated that the overall calorie intake of carbohydrates and fats was in close correlation with nutritional supplementation (Jaraari *et al.,* 2010; Tsai *et al.,* 2004).

Most patients who have asymptomatic gallstones don't grow to have symptoms or complications related to gallstones, increasing evidences suggest that stones in gallbladder can be accompanied by an increase in overall mortality and other health issues like cancer, cardiovascular disease and non-alcoholic fatty liver disease (Evan *et al.,* 2016). The techniques of diagnosis includes abdominal ultrasound which could easily be done by general practitioners (Eslami *et al.,* 2007) as well as cholescintigraphy (Krishnamurthy and Krishnamurthy, 1992; Sharma *et al.,* 2015), computed tomography (CT) and magnetic resonance cholangio pancreatography (Portincasa *et al.,* 2016).

The Fourier Transform Infrared Spectroscopy (FT-IR) technique, commonly employed in chemistry, has recently been utilized for structural examination in biomedical studies due to its benefits compared to other methods of analysis (Bazin and Daudon, 2012; Bunaciu *et al.,* 2014). This spectral method is extremely fast, requiring just a very low amount of sample, is very accurate and sensitive, giving repeatable outcomes (Yoo *et al.,* 2008). FT-IR is an appropriate technique for analysis of gallstones (Ishida *et al.,* 1987, Kleiner *et al.,* 2002). Hence, we have analyzed the gallstones composition utilizing FT-IR spectroscopy in this current research work.

## Materials and methods

*Number of subjects*

Forty (40) specimens of the gallstones were taken from the infected peoples who were admitted and cured in the Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, Nawabshah, Sindh, Pakistan. From these forty samples 07 were males with age ranges from 20-60 years and 33 were females with age ranges from 20-65 years. The ratio of male to female was found to be 1:4.7 as presented in Fig. 1-2. and Table 2. The specimens of gallstones were found with black, whitish yellow, brown, green and greenish brown colours. All the affectless were the resident of same district and having low socio-economic background.

**Table 1.** FT-IR absorption bands of components of gallstones ($cm^{-1}$).

| Types of gallstones | Cholesterol | Calcium carbonate | Calcium bilirubinate |
|---|---|---|---|
| Our Study | | | |
| Present study | 3415, 2937, 2869, 1468, 1371, 1058 | 1418, 1452, 1474, 871, 854 | 3397, 1658, 1571, 1443, 1250, 697 |
| Literature | | | |
| Kleiner *et al.*, 2002 | 3398, 2933, 2866 1463, 1376, 1056 | 1464, 875 | 1661, 1640, 1575 |
| Channa *et al.*, 2008 | 2929, 2899, 2865, 1463, 1054 | 1338, 854 | 3390, 1660, 1435 |
| Ha *et al.*, 2018 | 3410, 2934, 2853, 1458, 1373, 1055 | 1464, 1458, 1420, 872, 855 | 3398, 1663, 1624, 1566, 1447, 1251, 699 |

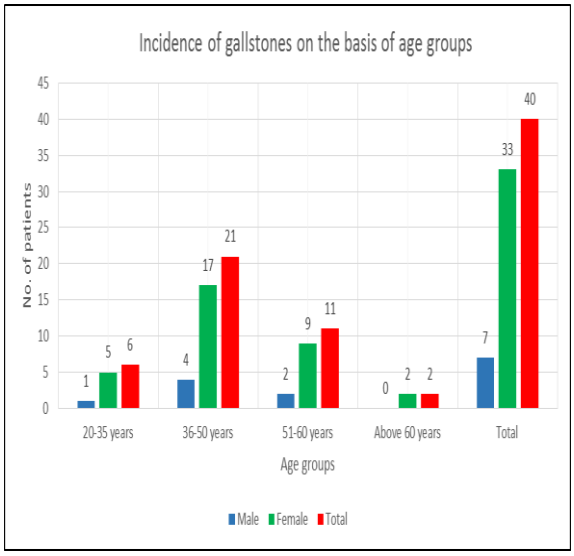


**Fig. 1.** Incidence of gallstones on the basis of age groups.

*Examination of samples*

Gallstones, which are being investigated, were collected from infected people. Forty (40) subjects were evaluated during 2018-2019. Samples collected were desiccated with a sterile gauze, the biles and remains were cleaned away by $H_2O$ de-ionised and were left to dry on silica gel over a week. These samples were divided into 4 equally spaced pieces and a piece of each was entirely crushed using a crusher and then transported to a test-tube and dissolved by mixing them with different solvents according to the components' nature and then investigated through FT-IR. The mass of each stone was determined using an analytical balance. Several physical characteristics of the gallstones, like their numbers, shapes, sizes etc were recorded.

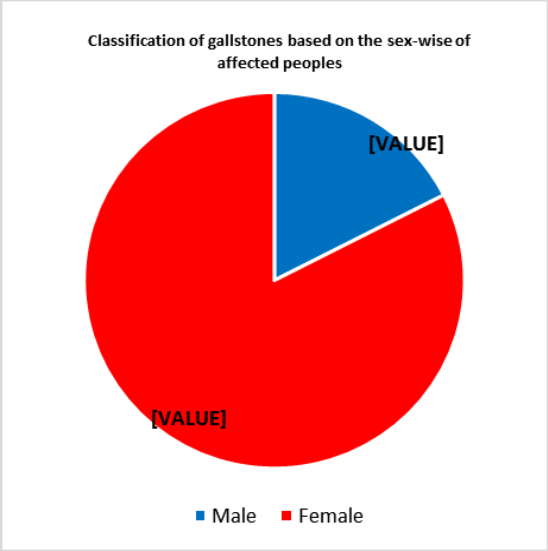


**Fig. 2.** Classification of gallstones based on the sex-wise of affected peoples.

## Results and discussion

In our work gallstones (total 40) were found in the age ranges from 20-60 years for male (07) patients and from 20-65 years for female (33) patients as shown in Fig. 2. and Table 2. The greatest ratio of gallstones was found 21/40 in the age ranges 36-50 years of affected peoples followed by 06/40 in the age group 20-35, 11/40 in the age group 51-60 and 02/40 in the age group above 61 as shown in Fig. 1. and Table 2. The occurrence of gallstones was higher in females than males and sex ratio was found to be 1:4.7 male to female as shown in Table 2.

**Table 2.** Incidence and classification of gallstones on the basis of age groups and sex-wise of affected peoples.

| Incidence of gallstones on the basis of age groups | | | | | |
|---|---|---|---|---|---|
| Age in groups | 20-35 | 36-50 | 51-60 | Above 60 | Total |
| Male | 01 | 04 | 02 | 00 | 07 |
| Female | 05 | 17 | 09 | 02 | 33 |
| Total | 06 | 21 | 11 | 02 | 40 |
| Sex-wise classification | | | | | |
| Gender | | Male | Female | | Total |
| Number of patients | | 07 | 33 | | 40 |
| %age | | 17.5% | 82.5% | | 100% |
| Male: Female Ratio | | | 1:4.7 | | |

The size and weight of gallstones vary and the size and weight of pure cholesterol stones were calculated as 0.36-2.27cm and 0.231-0.964g respectively while calcium carbonate stones were as 0.8-2.1cm and 0.305-0.646g respectively and 1.4-2.2cm size and 0.307-0.853g weight for calcium billirubinate gallstones were measured as shown in Fig. 3-4. and Table 3. In current work the cholesterol gallstone was the most common type of gallstones. The 28 (70%) of sample stones were detected as pure cholesterol gallstones while pure calcium carbonate were 05 (12.5%) and 07 (17.5%) were calcium bilirubinate out of 40 specimens of gallstones as shown in Table 3. The 28 gallstones were irregular and 12 were round in shape. Moreover, From 40 gallstone specimens 29 were found with smooth surfaces while 11 were with rough surfaces.

**Table 3.** Morphological characters like size and weight of gallstones.

| Types of stones | No. of specimens | Size of stones | | Weight of stones | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| Pure Cholesterol | 28 (70.0%) | 0.36cm | 2.27cm | 0.231g | 0.964g |
| Pure Calcium Carbonate | 05 (12.5%) | 0.8cm | 2.1cm | 0.305g | 0.646g |
| Calcium Bilirubinate | 07 (17.5%) | 1.4cm | 2.2cm | 0.307g | 0.853g |

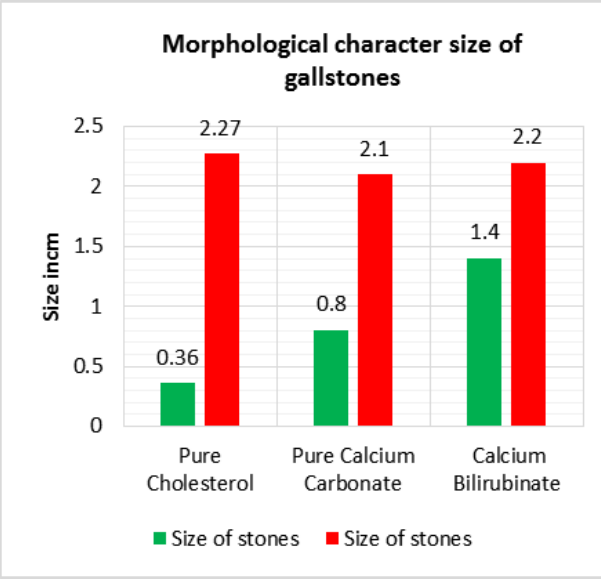


**Fig. 3.** Morphological character size of gallstones.

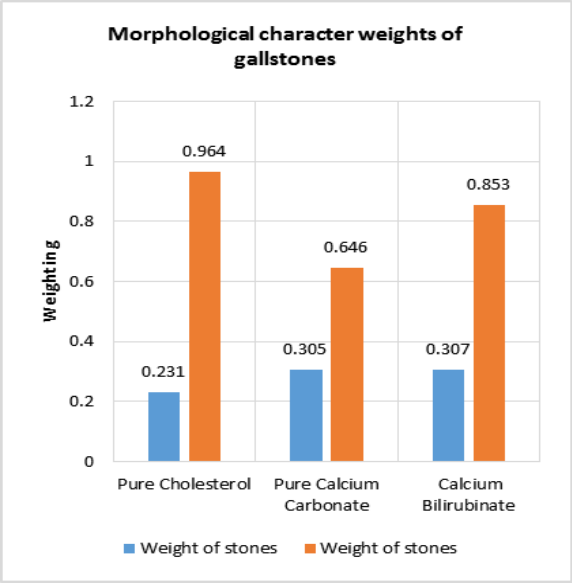


**Fig. 4.** Morphological character weights of gallstones.

The collected samples were analyzed using FT-IR and bands 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 1470cm$^{-1}$. The cholesterol presence in gallstones was identified by a high absorption range of O-H stretching at 3398cm$^{-1}$, band of stretching vibration for C-H at 2934cm$^{-1}$, band of deformation for C-H at 1466cm$^{-1}$ and absorption sharp peak at 1056cm$^{-1}$, which could be attributed to the cholesterol deformation of the ring. The strong characteristic peak results from symmetric $CH_2$ stretching vibrations is at 2901cm$^{-1}$. The cholesterol doublets band at 1378 and 1365cm$^{-1}$ are for bending vibration of ($CH_2$ and $CH_3$). The Calcium Carbonate FT-IR spectrum has broad absorption peaks at 1420-1480cm$^{-1}$ and sharp absorption peaks at 872 and 855cm$^{-1}$ as shown in Table 1. In gallstones the calcium carbonate is easily identified by utilizing FTIR spectroscopy since the characteristics bands of absorption at 855 and 872cm$^{-1}$ don't influence the cholesterol or calcium bilirubate absorption bands. The calcium carbonate may be identified along with calcium bilirubate, cholesterol or both and the colour of such stones in all cases is light brown to dark brown. The bands for the constituents of stones in gallbladder were in accordance with the reported literature as presented in Table 1.

**Conclusion**

From this study work it is concluded that cholesterol stone was the most common type of gallstones and the greatest ratio of gallstones was found 21/40 in the age ranges 36-50 years of affected peoples. The occurrence of gallstones was higher in females than males and sex ratio was found to be 1:4.7 male to female.

**References**

**Bassi N, Del Favero G, Meggiato T, Scalon P, Ghiro S, Molin M, Pilotto A, Vigneri S, Savarino V, Mela GS, Di Mario F.** 1994. Are morphology and composition of gallstones related? An X-ray diffraction study. Current therapeutic research **55(10),** 1169-1175.

**Bazin D, Daudon M.** 2012. Pathological calcifications and selected examples at the medicine–solid-state physics interface. Journal of Physics D: Applied Physics **45(38),** 383001-10.
DOI: http://dx.doi.org/10.1088/0022-3727/45/38/3830

**Bunaciu AA, Fleschin S, Aboul-Enein HY.** 2014. Biomedical investigations using Fourier transform-infrared microspectroscopy. Critical Reviews in Analytical Chemistry **44(3),** 270-276.

**Channa NA, Khand FD, Bhanger MI.** 2008. Analysis of Human Gallstones by FTIR. Malaysian Journal of Analytical Sciences **12(3),** 552-560.

**Channa NA, Khand FD, Khand TU, Leghari MH, Memon AN.** 2007. Analysis of human gallstones by Fourier Transform Infrared (FTIR). Pakistan Journal of medical sciences **23(4),** 546-550.

**Eslami G, Nourouzi J, Falah F, Goudarzi H, Hakemi VM, Jahangiri S.** 2007. Detection of bacteria responsible for gallbladder inflammation and gallstones. Iranian Journal of Clinical Infectious Diseases **2(3),** 139-141.

**Evan T, Lee SP, Ko CW.** 2016. Gallstones: new insights into an old story. F1000 Research **5,** 1-8.
DOI: http://dx.doi.org/10.12688/f1000research.8.1.

**Gurusamy KS, Davidson BR.** 2014. Gallstones. Bmj **348,** 2669-2674.

**Ha BJ, Park S.** 2018. Classification of gallstones using Fourier-transform infrared spectroscopy and photography. Biomaterials research **22(1),** 18-25.

**Ishida H, Kamoto R, Uchida S, Ishitani A, Ozaki Y, Iriyama K, Tsukie E, Shibata K, Ishihara F, Kameda H.** 1987. Raman Microprobe and Fourier Transform-Infrared Microsampling Studies of the Microstructure of Gallstones. Applied Spectroscopy **41,** 407-412.

**Jaraari A, Jagannadharao P, Patil T, Hai A, Awamy H, El Saeity S, Abdel Kafi E, El-Hemri M, Tayesh M.** 2010. Quantitative analysis of gallstones in Libyan patients. Libyan Journal of Medicine **5(1),** 4627-4632.

**Kalloo AN, Kantsevoy SV.** 2001. Gallstones and biliary disease. Primary Care **28(3),** 591-606.

**Kleiner O, Ramesh J, Huleihel M, Cohen B, Kantarovich K, Levi C, Polyak B, Marks RS, Mordehai J, Cohen Z, Mordechai S.** 2002. A comparative study of gallstones from children and adults using FTIR spectroscopy and fluorescence microscopy. BMC Gastroenterology **2(3),** 1-14.

**Kratzer W, Mason RA, Kächele V.** 1999. Prevalence of gallstones in sonographic surveys worldwide. Journal of clinical ultrasound **27(1),** 1-7.

**Krishnamurthy S, Krishnamurthy GT.** 1992. Gallbladder ejection fraction: a decade of progress and future promise. The Journal of Nuclear Medicine **32(4),** 542-544.

**Le Bail B, Balabaud C, Bioulac-Sage P.** 1992. Anatomy and structure of the liver and biliary tree. In Hepatobiliary Diseases 1-38.
DOI: https://doi.org/ 10.1007/978-3-642-76802-6_1

**Maurer KJ, Carey MC, Fox JG.** 2009. Roles of infection, inflammation, and the immune system in cholesterol gallstone formation. Gastroenterology **136(2),** 425-440.

**Portincasa P, Di Ciaula A, De Bari O, Garruti G, Palmieri VO, Wang DH.** 2016. Management of gallstones and its related complications. Expert Review of Gastroenterology & Hepatology **10(1),** 93-112.

**Reshetnyak VI.** 2012. Concept of the pathogenesis and treatment of cholelithiasis. World Journal of Hepatology **4(2),** 18-34.

**Sharma R, Soy S, Kumar C, Sachan SG, Sharma SR.** 2015. Analysis of gallstone composition and structure in Jharkhand region. Indian Journal of Gastroenterology **34(1),** 29-37.

**Sikkandar S, Jayakumar S, Gunasekaran S, Renugadevi TS, Alwar B.** 2011. Study on the analysis of human gallstones using Fourier transform infrared spectroscopic technique. Int J Chem Tech Res **3(1),** 149-54.

**Tsai CJ, Leitzmann MF, Hu FB, Willett WC, Giovannucci EL.** 2004. A prospective cohort study of nut consumption and the risk of gallstone disease in men. American Journal of Epidemiology **160(10),** 961-968.

**Yoo EH, Oh HJ, Lee SY.** 2008. Gallstone analysis using Fourier transform infrared spectroscopy (FT-IR). Clinical Chemical Laboratory Medicine **46(3),** 376-381.

**Zhou XS, Shen GR, Wu JG, Li WH, Xu YZ, Weng SF, Soloway RD, Fu XB, Tian W, Xu Z, Shen T.** 1997. A spectroscopic study of pigment gallstones in China. Biospectroscopy **3(5),** 371-380.