



Investigation of gallstones laproscopically removed from the patients using FTIR

Nafeesa Khatoon Shahani¹, Ghulam Abbas Shar^{*1}, Gul Afshan Soomro¹,
Muhammad Bachal Korai¹, Nisar Abbas Shar², Rafique Abbas Shar²

¹*Institute of Chemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan*

²*Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad,
Nawabshah, Sindh, Pakistan*

Article published on April 19, 2020

Key words: Chemical composition, Gallstone, Mixed stone, Cholesterol stone, FTIR

Abstract

The current research work was mainly focused on the determination of the composition of gallstones laproscopically removed from the patients in Khairpur Sindh, Pakistan. Analysis of constituents of gallstone in patients is carried out by FTIR from patients hospitalized in Khairpur Medical College Civil Hospital and from Gambat Institute of Medical Sciences Hospital during 2018 to 2019. A total of 100 (20 males and 80 females) samples of stones laproscopically removed from patients were collected and studied. Cholesterol, pure calcium carbonate, cholesterol + calcium carbonate, cholesterol + bilirubin and calcium bilirubinate stones were found in 65 (65%), 7 (7%), 10 (10%), 12 (12%) and 6 (6%) patients respectively. It is clear from examination of gallstones with Fourier Transform Infrared (FTIR) Spectroscopy that in gallstones the predominant component was cholesterol either only in cholesterol stones or in mixed stones in combination with other components.

***Corresponding Author:** Ghulam Abbas Shar ✉ gabbas.shar@salu.edu.pk

Introduction

Gallstone disease is becoming the major health problem on the globe (Harding, 1964; Kern, 1983; Sharma *et al.*, 2016; Vaibhaw H, Girikumar *et al.*, 2019). In recent years number of affecties is increasing day by day in Pakistan (Channa *et al.*, 2004; Channa *et al.*, 2008), especially in upper sindh. Gallstone results in the complex biomineralized deposition in gallbladder (Kravets and Moskalenko, 2016; Wentrup-Byrne *et al.*, 1997). The constituents of bile are primarily concerned with gallstones forming in the gallbladder (Tanaka *et al.*, 2018).

The gallstones are smaller in sizes from 1cm³ to 13cm³. The bile as a stream, blocked within a duct, causes the liver to flow to the small intestine (Jaiswal and Chamarthi, 2016). Which includes the liver ducts that carry the bile away from the liver, a duct carrying the bile out of the gallbladder and into the gallbladder is also the cystic duct and a duct carrying the bile to the small intestine. As the bile goes to the small intestine, which is the bile common to cystic and hepatic ducts. Inflammation of the gallbladder could be induced by entrapment of bile within a duct because of the infection, it is the duct of the pancreatic that is responsible for enzyme in the digestive tract. Pain in gallstones in the upper right abdomen is usually an infection by eating large amounts of fatty foods, stomach pain associated with vomiting, nausea, indigestion or swelling of the abdomen (Bianco *et al.*, 2018). Many different types of gallstones exist and each one has a distinct origins (Khan *et al.*, 2017).

The composition of gallstones is different so, on the basis of this gallstones are classified as: pure cholesterol, pigment and mixed gallstones (Idris *et al.*, 2014). Cholesterol stones are lemon yellow to dark green (brown) in colour and contain 70% - 80% of cholesterol by weight (Fauziah *et al.*, 2016; Iqbal *et al.*, 2019). Pigment stones are brown or black and contain various salts of bilirubin with less than 20% of cholesterol by weight (Ravichandran *et al.*, 2017).

Calcium palmitate is major constituent of brown stone while calcium phosphate and calcium carbonate both are of black stones. And 20% - 80% of cholesterol is in mixed stones. There are many factors such as obesity (Amaral *et al.*, 1985; Chandran *et al.*, 2014; Jørgensen, 1989), diabetes mellitus (Liu *et al.*, 2004), psychological stress (Geetha, 2002), a diet rich in saturated fats (Jonnalagadda *et al.*, 1995; Tsai *et al.*, 2014), having legumes (Nervi *et al.*, 1989; Thijs and Knipschild, 1990), tomato, palak, uncooked green chilies (Marteau *et al.*, 1980; Noorani, 2006) may cause disease of the pure cholesterol stones/ Gallstones. The treatment for gall bladder removal is laparoscopic surgery. This procedure is generally called cholecystectomy. Opened surgical treatment is also an option. The redundant organ of human beings is the gallbladder if removed, then bile flows into the small intestine directly. Several dietetic and house-based medications have been made to deal with gallstones as well as to prevent the signs of gallstones, but have not yielded positive outcomes. In present study, the compositions of 100 samples of gallstones are analyzed with Fourier Transform Infrared Spectroscopy.

Materials and methods

Patient population

Total 100 patients of gallstones were under examination, 80 females (age 20 to 65 years old) and 20 males (age 20 to 60 years old) and their Female: Male ratio was 4:1 as shown in Table 1. All patients belong to same low socio-economic status, living in upper Sindh, Pakistan. They took easily available food like various types of vegetable foods, different types of pulses, rice, wheat meal and some meat.

Stone Analysis

Gallstones, material under study, were collected from patients through laproscopically. 100 patients were examined during the period from 2018-2019. The samples obtained were dried by placing them on sterile gauze, bile and debris were removed with deionized water, and then left them over silica gel to be dried for a week. Gallstones were taken and cut into four equal pieces and a quarter piece of each was changed into powder completely then transferred in test tube and dissolved in different solvents

depending on nature of their constituents and then was analyzed by FTIR.

Results and discussion

Fig. 1, 3 and 6 show the FTIR spectrum for cholesterol standard, pure bilirubin standard and CaCO₃ standard respectively.

Table 2 shows that 20% of patients were with below than 25% cholesterol gallstone. 13% of them were cholesterol free. 53% were with 100% cholesterol, 12% with equal or greater than 80% but less than 100% of cholesterol, 3% with ≥ 50 and < 80% and 12% of patients had 25-49% of cholesterol present in gallstones. Most of the gallstones were with cholesterol.

Table 1. Distribution of patients according to their gender.

Gender	Number of patients
Female	80
Male	20
Female:Male Ratio	4:1

Table 2. % age by weight of cholesterol in gallstones removed from 100 patients.

Cholesterol % by weight	No. of patients	% age of patients
0	13	13
< 25	7	7
≥ 25 and < 50	12	12
≥ 50 and < 80	3	3
≥ 80 and < 100	12	12
= 100	53	53

Table 3 shows the gallstone types and IR bands of components of gallstone. 65/100 (65%), 7/100 (7%)

and 6/100 (6%) were found as pure cholesterol, pure CaCO₃ and calcium bilirubinate stones respectively. Others 10/100 (10%) and 12/100 (12%) were observed as mixtures of cholesterol with CaCO₃ and cholesterol with bilirubin respectively. Strong bands at about 2929.40, 1462.43 and 1054cm⁻¹ were observed for Cholesterol (Fig. 2). Similarly for bilirubin the diagnostic bands (Fig. 4) were 1683 and 1607 and 1246; for calcium bilirubinate (Fig. 5) were 3390, 1621.79 and 1450.17; and for CaCO₃ (Fig. 7) were 1406.22 and 859.92cm⁻¹. The different composition of central part, the periphery and the whole stone (parts of gallstone) were analyzed. Cholesterol and bilirubin were observed in the highest concentration at periphery. The highest concentration of cholesterol was seen in each part of gallstones.

Table 3. Type, occurrence, and FTIR bands of principal components observed in gallstones.

Types of gallstones	No. of sample	Principal FTIR bands examined	Literature values
Pure Cholesterol	65	2929.40, 2892.62, 2859.93, 1462.43, 1054	2925 (CH ₂ and CH ₃ asymmetric stretching), 1460 (CH ₂ and CH ₃ bending), 1050 (C-C stretching) [Zhou <i>et al.</i> , 1997]
Pure calcium carbonate	7	1406.22, 859.92	1481, 855 [Kliener <i>et al.</i> , 2002]
Cholesterol + calcium carbonate	10	-do-	Same 1481, 855 [Kliener <i>et al.</i> , 2002]
Cholesterol + bilirubin	12	1683, 1607, 1246	Identical to pure cholesterol and 1670, 1640 (OC=O stretching), 1575 (C=C stretching)
Calcium bilirubinate	6	3390, 1621.79, 1450.17	3410 (CH ₂ and CH ₃ asymmetric stretching), 1380 (CH ₂ and CH ₃ bending)

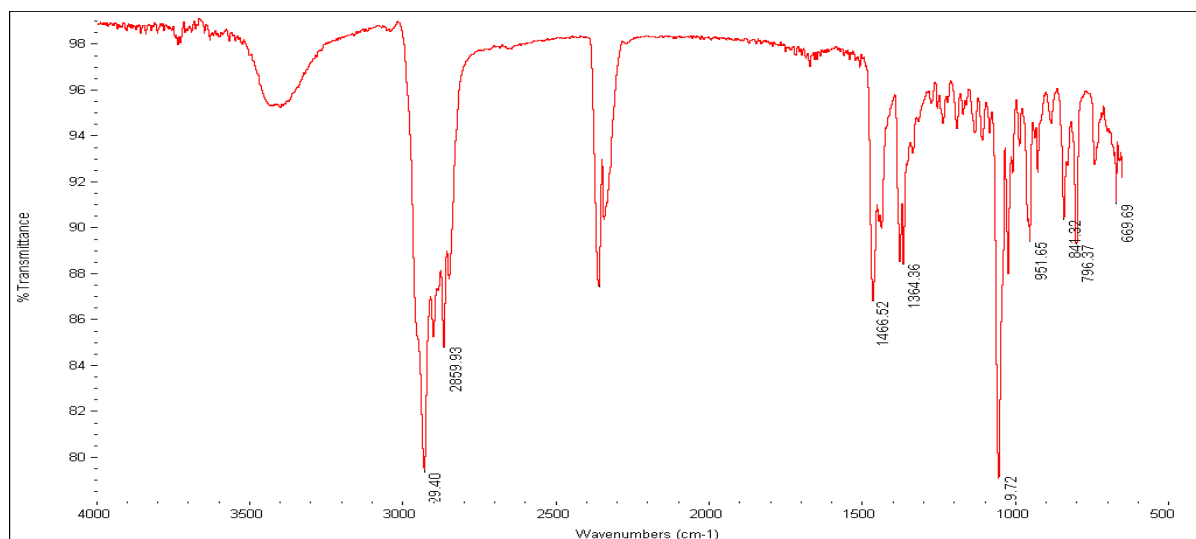


Fig. 1. FT-IR Spectrum of Cholesterol standard.

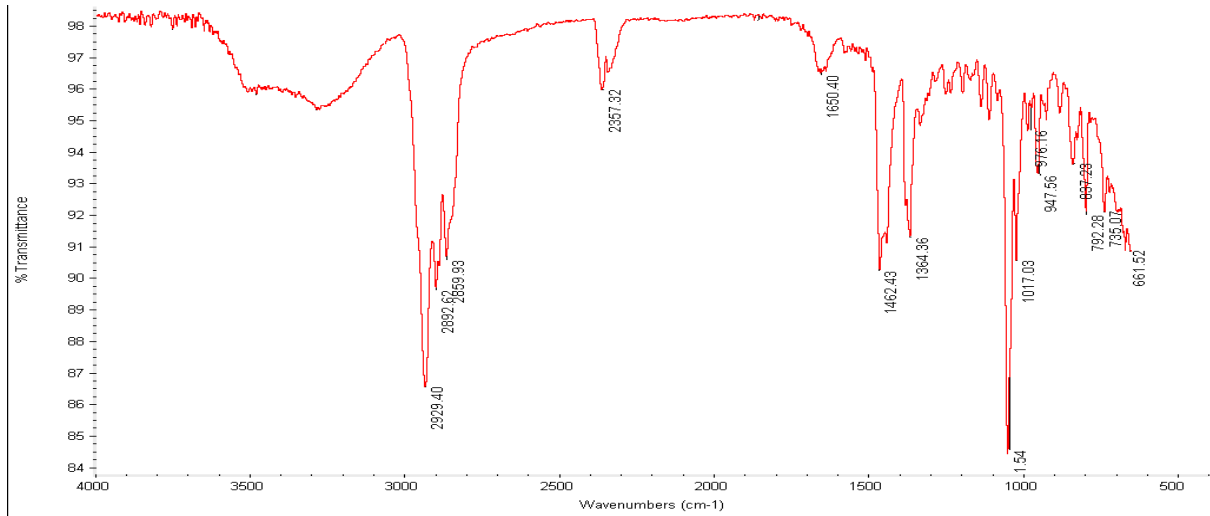


Fig. 2. FT-IR Spectrum of Cholesterol Gallstone.

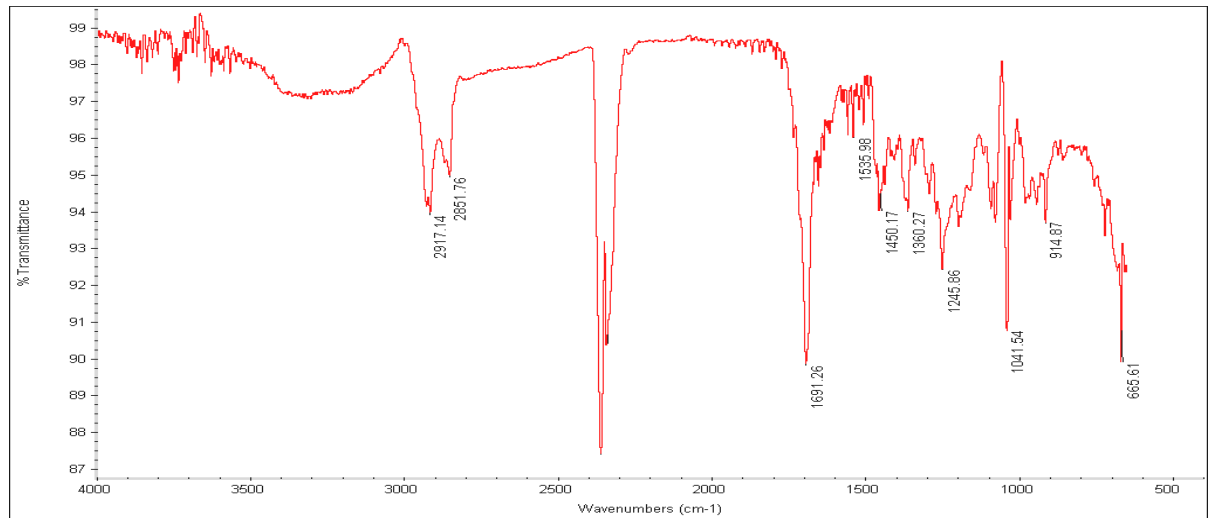


Fig. 3. FT-IR Spectrum of pure Bilirubin standard.

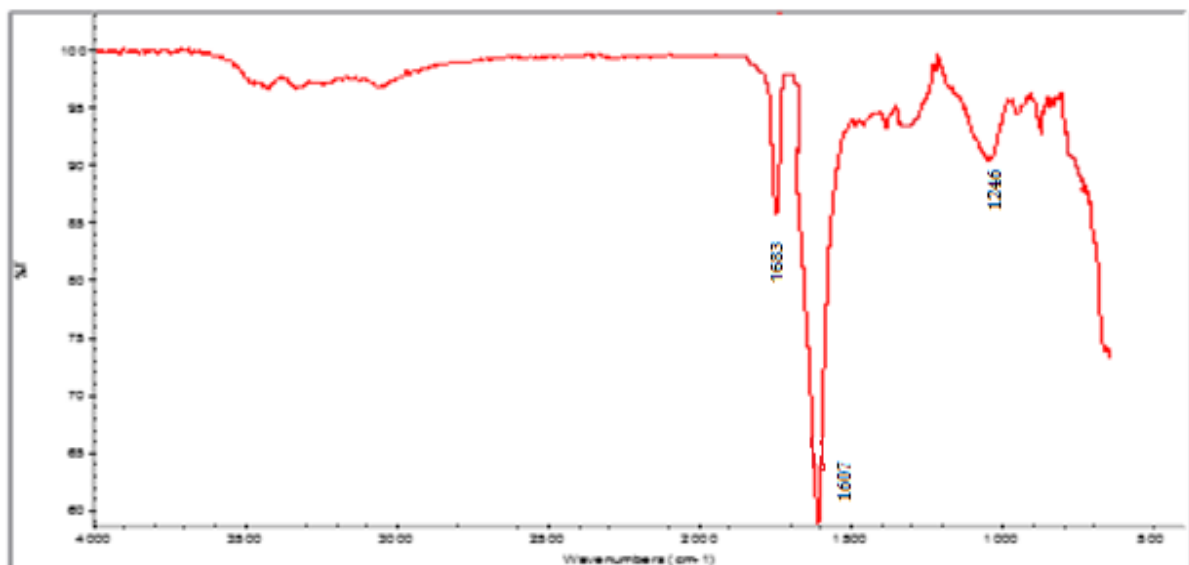


Fig. 4. FT-IR Spectrum of Bilirubin sample.

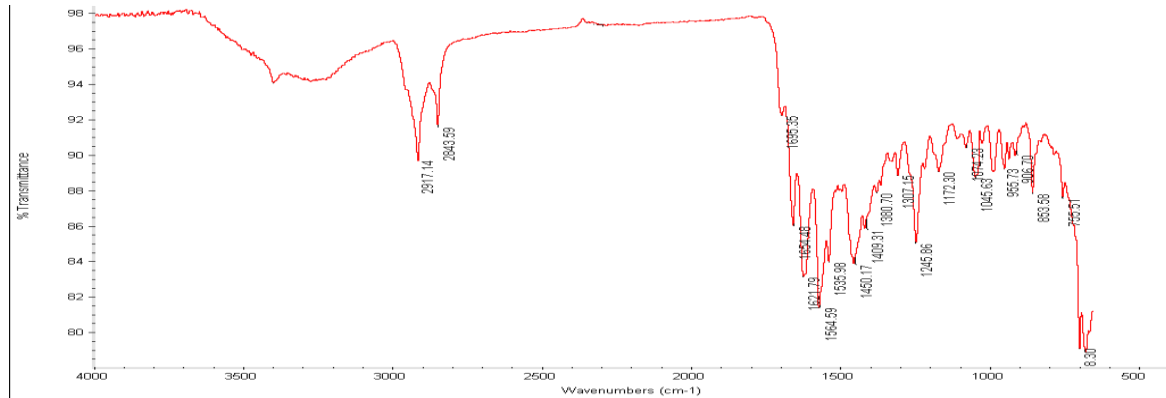


Fig. 5. FT-IR Spectrum of Calcium Bilirubin Gallstone.

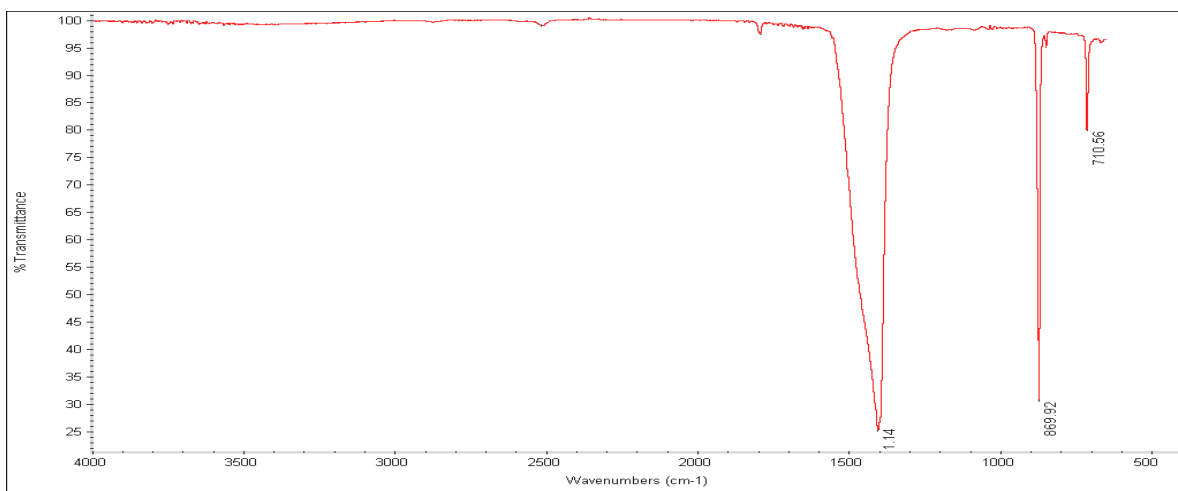


Fig. 6. FT-IR Spectrum of Pure Calcium Carbonate standard.

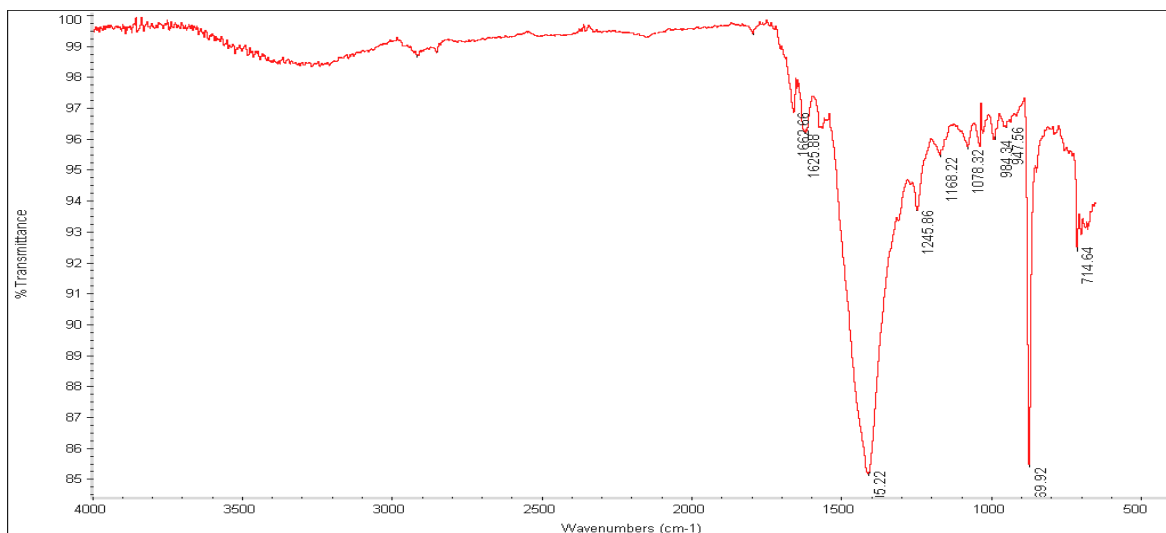


Fig. 7. FT-IR Spectrum of Pure Calcium Carbonate Gallstone.

In the present study various bands for the components of gallstones were according to reported literatures (Kim *et al.*, 1999; Wentrup-Byrne *et al.*,

1997). Most of the cholesterol stones were with pigments and clear crystals of cholesterol on the centre and periphery respectively and some with

radial structure (Kaufman *et al.*, 1994). Cholesterol and black pigment gallstones were not chemically alike. Fatty acids, bilirubinate and negligible amount of calcium carbonate were the constituents of black pigment gallstones. Black pigment gallstones were tiny, deep brown or black and irregular.

The finding shows that pure cholesterol stones were in major proportion (65%) and also verify the already work done on it in Pakistan that pure cholesterol gallstones are common than others (Channa *et al.*, 2008; Khand, 1997). There are many factors such as obesity (Amaral *et al.*, 1985; Chandran *et al.*, 2014; Jørgensen, 1989), diabetes mellitus (Liu *et al.*, 2004), psychological stress (Geetha, 2002), a diet rich in saturated fats (Jonnalagadda *et al.*, 1995; Tsai *et al.*, 2014), having legumes (Nervi *et al.*, 1989; Thijs and Knipschild, 1990), tomato, palak, uncooked green chilies (Marteau *et al.*, 1980; Noorani, 2006) may cause disease of the pure cholesterol stones/ Gallstones.

Conclusion

It is concluded from this study that gallstones removed from the patients of district Khairpur, Sindh, Pakistan, the cholesterol is predominant component and it is either alone or with bilirubin or CaCO₃.

References

Amaral JF, Thompson WR. 1985. Gallbladder disease in the morbidly obese. *American Journal of Surgery* **149(4)**, 551-557.

Bianco G, Frongillo F, Agnes S, Nure E, Silvestrini N. 2018. Gallbladder agenesis: A case report and brief review. *Annals of hepato-biliary-pancreatic surgery* **22(3)**, 292-295.

Chandran AP, Sivarajan R, Srinivasan V, Srinivas M, Jayanthi V. 2014. Risk profile for gallstone disease in southern Indian population: is there anything new?. *Indian Journal of Gastroenterology* **33(3)**, 254-257.

Channa NA, Khand FD, Bhanger MI, Laghari MH. 2004. Surgical incidence of cholelithiasis in

Hyderabad and Adjoining areas (Pakistan). *Pakistan Journal of Medicinal Sciences* **20(1)**, 13-17.

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

Fauziah CI, Zaibunnisa AH, Osman H, Wan Aida WM. 2016. Physicochemical analysis of cholesterol-reduced egg yolk powder and its application in mayonnaise. *International Food Research Journal* **23(2)**, 575-582.

Geetha A. 2002. Evidence for oxidative stress in the gall bladder mucosa of gall stone patients. *Journal of biochemistry, molecular biology, and biophysics: JBMBB: the official journal of the Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB)* **6(6)**, 427-432.

Harding AJ. 1964. Gallstones: causes and treatments. In William Heinemann Medical Books, London.

Idris SA, Elsiddig KE, Hamza AA, Hafiz MM, Shalayel MH. 2014. Extensive quantitative analysis of gallstones. *International Journal of clinical medicine* **2014(5)**, 42-50.

Iqbal MN, Iqbal MA, Javaid R, Abbas MW. 2019. Gall stones: a fundamental clinical review. *International Journal of Research in Medical Sciences* **7(7)**, 2869.

Jaiswal S, Chamarthi S. 2016. Bile duct stones: making the radiologic diagnosis. In *Multidisciplinary Management of Common Bile Duct Stones* (pp.17-25). Springer, Cham.

Jonnalagadda SS, Trautwein EA, Hayes KC. 1995. Dietary fats rich in saturated fatty acids (12: 0, 14: 0, and 16: 0) enhance gallstone formation relative to monounsaturated fat (18: 1) in cholesterol-fed hamsters. *Lipids* **30(5)**, 415-424.

Jørgensen T. 1989. Gall stones in a Danish population. Relation to weight, physical activity, smoking, coffee consumption, and diabetes mellitus. *Gut* **30(4)**, 528-534.

Kaufman HS, Magnuson TH, Pitt HA, Frasca P, Lillemoe KD. 1994. The distribution of calcium salt precipitates in the core, periphery and shell of

- cholesterol, black pigment and brown pigment gallstones. *Hepatology* **19(5)**, 1124-1132.
- Kern F.** 1983. May. Epidemiology and natural history of gallstones. In *Seminars in liver disease* **3(2)**, 87-96. © 1983 by Thieme Medical Publishers, Inc.
- Khan M, Kazi TG, Afridi HI, Bilal M, Akhtar A, Khan S, Kadar S.** 2017. Variation of calcium, copper and iron levels in serum, bile and stone samples of patients having different types of gallstone: A comparative study. *Clinica Chimica Acta* **471**, 254-262.
- Khand FD.** 1997. Cholelithiasis in southern Sindh. *Pakistan: incidence and composition of gallstones*, *Specialist Q* **13(3)**, 263-70.
- Kim MH, Lim BC, Myung SJ, Lee SK, Ohrr HC, Kim YT, Roe IH, Kim JH, Chung JB, Kim CD, Shim CS.** 1999. Epidemiological Study on Korean Gallstone Disease (A Nationwide Cooperative Study). *Digestive diseases and sciences* **44(8)**, 1674-1683.
- Kleiner O, Ramesh J, Huleihel M, Cohen B, Kantarovich K, Levi C, Polyak B, Marks RS, Mordechai 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.
- Kravets OV, Moskalenko RA.** 2016. Morphological analysis of porcelain gallbladder (Doctoral dissertation, «East West» Association for Advanced Studies and Higher Education GmbH).
- Liucm, Tung TH, Liu JH, Lee WL, Chou P.** 2004. A community-based epidemiologic study on gallstone disease among type 2 diabetics in Kinmen, Taiwan. *Digestive Diseases* **22(1)**, 87-91.
- Marteau C, Montet JC, Gerolami A.** 1980. Dietary caloric intake and cholesterol lithiasis. *Medecine & chirurgie digestives* **9(5)**, 397.
- Nervi F, Covarrubias C, Bravo P, Velasco N, Ulloa N, Cruz F, Fava M, Severín C, Del Pozo R, Antezana C, Valdivieso V.** 1989. Influence of legume intake on biliary lipids and cholesterol saturation in young Chilean men: identification of a dietary risk factor for cholesterol gallstone formation in a highly prevalent area. *Gastroenterology* **96(2)**, 825-830.
- Noorani N.** 2006. The epidemiological and biochemical aspects involved in the pathogenesis of human gallstones (Cholelithiasis) in Southern Sindh, Pakistan (Doctoral dissertation, Ph. D. thesis, University of Sindh).
- Ravichandran G, Lakshminarayanan G, Gopinath D, Arumugam S.** 2017. Characterisation of Gallstones Using Fourier Transform Infrared Spectroscopy. *Journal of Applied Physics* **9(4)**, 10-18.
- Sharma RK, Sonkar K, Sinha N, Rebala P, Albani AE, Behari A, Reddy DN, Farooqui A, Kapoor VK.** 2016. Gallstones: a worldwide multifaceted disease and its correlations with gallbladder carcinoma. *PloS one* **11(11)**, 1-14.
- Tanaka H, Imasato M, Yamazaki Y, Matsumoto K, Kunimoto K, Delpierre J, Meyer K, Zerial M, Kitamura N, Watanabe M, Tamura A.** 2018. Claudin-3 regulates bile canalicular paracellular barrier and cholesterol gallstone core formation in mice. *Journal of Hepatology* **69(6)**, 1308-1316.
- Thijs C, Knipschild P.** 1990. Legume intake and gallstone risk: results from a case-control study. *International journal of epidemiology* **19(3)**, 660-663.
- Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL.** 2004. The effect of long-term intake of cis unsaturated fats on the risk for gallstone disease in men: a prospective cohort study. *Annals of internal medicine* **141(7)**, 514-522.
- Vaibhaw H, Girikumar E, Mandke DH, Sinha RNP.** 2019. A Histomorphological Study on Gall Bladder Lesions at a Tertiary Care Centre. *Annals of International Medical and Dental Research* **5(2)**, 39-41.
- Wentrup-Byrne E, Chua-Anusorn W, St. Pierre TG, Webb J, Ramsay A, Rintoul L.** 1997. A Spectroscopic study of Thalassemic Gallstones. *Biospectroscopy* **3(5)**, 409-416.
- 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.