

Original research article**Cross Sectional Study of Bile Bacteriology in Calculus Disease of Gall Bladder and their Sensitivity Pattern****Dr. Abhay Kumar¹, Dr. Neha Singh², Dr. Rajeev Kumar³**¹Senior Resident, Department of Medicine, AIIMS Patna²Senior Resident, Department of Surgery, AIIMS Patna³Senior Resident, Department of Medicine, AIIMS Patna**Corresponding Author: Dr. Rajeev Kumar****Abstract**

Background: Gallbladder disease is the commonest surgical problem and cholecystectomy is the most frequently performed operation. In spite of modern standards of pre-operative preparation and refinements in anesthetic and operative techniques, post-operative wound infections occur in quite a number of patients. With introduction of newer and costlier antibiotics for preventing post-operative wound infection¹.

Methods: 139 patients with gallstone disease who undergone cholecystectomy in All india institute of medical sciences Patna. Data related to the objectives of the study were collected. Adult patients undergoing elective or emergency cholecystectomy were taken for the study. clinical examination and underwent various investigations including complete blood counts, liver function tests, renal function tests, ECG, X-ray and ultrasonography.

Results: Most of the patients undergoing cholecystectomy were in the mean age group of 45-54 years, with ages ranging between 15 to 77 years, and this is consistent with the observations made by Ferzli (1991)⁵. In our series, females (105) outnumbered the males (34). This indicates a higher incidence of the gallbladder stone in females as compared to males in the respective age groups⁶.

Conclusion: Positive bile culture was a common finding in patients with acute cholecystitis in this study. Escherichia coli are one of the most common isolated bacteria followed by Pseudomonas aeruginosa and Staphylococcus aureus. The resistance to second- generation cephalosporins has increased while third and fourth-generation cephalosporins show better promise and may be used as the first line of preoperative prophylaxis in operations for gallbladder stone disease.

Keyword: Gallstone, Cholelithiasis, Cholecystectomy, Infection, Laparoscopic cholecystectomy.

Introduction

Thrombocytopenia is one of the most important haematological manifestation of many Cholecystitis, in its varied forms, is one of the most prevalent surgical entities of the modern industrialized world. The most common cause of cholecystitis and biliary colic ischolelithiasis, a thorough understanding of which is valuable in their management. Biliary calculus disease is

one of the most common disorders affecting the gastrointestinal tract and is important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west during the past century. The pathogenesis of gallstones is multifactorial. It varies according to the type of gallstones. Primarily gallstones can be divided into two major groups. First is a pure gallstone contributing 10% of gallstones. Second is a mixed and combined gallstone which accounts for 90% of gallstones. Mixed gallstones frequently associated with cholecystitis. In about ½ of the cases bacteria can be cultured from the gallbladder bile.⁸ Infective factor seems to be a major cause of formation of gallstones. Moynihan's aphorism that –gall stone is a tomb stone erected in the memory of the organism within it is true today.⁷ Evidence in favor of infection includes isolation of *E. coli*, *bacterium typhosum*, *streptococcus* from the gallbladder bile and from the centre of the gallstones.⁸ Slow growing actinomyces also have been recovered from the bile. These organisms reach the gallbladder via blood stream, from infective focus elsewhere in the body and by lymphatics also. Brown pigment gallstones occur as a result of infection. Bacteria are found within the calcium bilirubinate and protein matrix of brown pigment gallstones.⁹

Objectives

Antibacterial sensitivity of antibiotics on organisms isolated from the bile of patients with gall bladder stone disease undergoing cholecystectomy.

Review of Literature

The most prevalent complication of cholelithiasis is chronic cholecystitis usually necessitating cholecystectomy¹⁰. The biliary tract is usually sterile; however, if cholelithiasis is pigmented and cholestering, different microbes might be identified in and/or cultured from the bile or gallbladder wall¹¹. Microscopic examinations indicated that 20-50% of the patients with chronic cholecystitis have positive bile culture¹³. Different reasons for biliary tract infection have been presented, e.g. ascending infection due to reflux of duodenal contents, blood-borne infection and infection spread through the portal-venous channels. Ascending infection from the duodenum is thought to be the primary mechanism by which bacteria enter the bile¹³. Different microbes in the bile may be cause to post- cholecystectomy infections. Thus, understanding the most common organisms causing them and their antibacterial susceptibility pattern would be useful in prevention of these infections. The present study was carried out to achieve this aim. In one of the studies in Saudi Arabia, the most common organism isolated were *E coli* (28.1%), *Enterococcus faecalis* (15.6%) and *Pseudomonas aeruginosa* (9.4%)¹⁶. An Indian study showed, *E. coli* (45.07%) and *Klebsiella* (25.35%) as predominant among the aerobes and *Bacteroides fragilis* (58.82%) among the anaerobes¹⁴. In a study by Irfan Sattar et al 36% had positive bile culture with *E. coli* as commonest organism followed by *Klebsiella*, *Pseudomonas* and *Staphylococcus aureus*¹⁵. Cholelithiasis was studied by Malatani Tarek S and et al to determine the constituents by culturing the bile specimen and correlating with the wound infection. It has shown that mixed gallstones were the commonest type (58.5%), pigment stones (27%) and cholesterol stones (14.5%). Positive bile cultures were found in 41 patients (27%) and *Escherichia coli* was the commonest organism isolated. Maki expressed the classic theory of the pathogenesis of calcium bilirubinate gallstone formation in which he emphasized the role of infection of bile with stagnation and the enzymatic hydrolysis of bilirubin glucuronide into free bilirubin and glucuronic acid. This free unconjugated bilirubin which is insoluble in water then combines with calcium in the bile to produce a calcium bilirubinate matrix¹⁷. Stewart and co-workers demonstrated the presence of bacteria in the interior of most pigment gallstones¹⁸. the role of bacteria in the formation of gallstones. In their study bacteria were identified only within the calcium bilirubinate – protein matrix of brown pigment stones¹⁹. In a review of biliary bacteriology in 200 consecutive patients with gallstone

disease, Tabata and Nakyama found that more than 80% patients had evidence of bactibilia (defined as more than 10^5 colony – forming units/ml)²⁰. In comparing black and brown pigment stones Cetta et al found positive bile culture in 25% patients with black gallstones and 100% patients with brown pigment gallstones.¹⁵ Studies by Stewart et al and Smith et al from United States, states that bacteria were found in the majority of black and brown pigmented gallstones concluding that the bacterial infection is the primary factor in both black and brown pigment gallstone pathogenesis.¹⁸ Infection has been documented at the time of gallstone removal in more than 90% brown gallstones. Bile infection by E.Coli precedes rather than follows brown gallstone formation.¹⁹ Ballal et al. have shown anaerobes were sensitive to Cefotaxime, Metronidazole, Chloramphenicol, Cefazolin, and Tetracycline, and aerobes isolated to Ampicillin, Chloramphenicol, Streptomycin, Tetracycline, Gentamicin, and second generation Fluoroquinolones such as Ciprofloxacin and Norfloxacin²¹. 1924 – Findlay introduced the concept that failure of cholesterol to remain in solution was the critical factor initiating cholesterol gallstone formation.⁷ 1966 – Maki proposed that bacterial infection plays a key role in the pathogenesis of pigment gallstones.⁸



Figure 1: Oral cholecystogram showing gallstones in the gallbladder

Material and methods

139 patients with gallstone disease who undergone cholecystectomy in, All india institute of medical sciences Patna, Bihar. Study duration of two years. Data related to the objectives of the study were collected. Adult patients undergoing elective or emergency cholecystectomy were taken for the study. clinical examination and underwent various investigations including complete blood counts, liver function tests, renal function tests, ECG, X-ray and ultrasonography.

Inclusion criteria

All patients above 13 years of age, undergoing cholecystectomy, Both, elective and emergency cases who is undergoing cholecystectomy.

Exclusion criteria

All patients below 13 years of age, Those with gall bladder malignancy.

Bile is aspirated from the gallbladder of the patient who underwent opencholecystectomy using a sterile syringe (10ml) .

In case of laparoscopic cholecystectomy, bile is collected from excised gall bladder. The sample is put in sterile bottle and is kept in the freezer before it is transferred to laboratory. In the laboratory the bile sample is put over peptone or glucose broth under aerobic condition and the results read after 48 hours for growth of organisms.

Results

In our present study 139 cases with calculus cholecystitis or chronic cholelithiasis with age group of 21 to 60 years are observed. Out of 139 cases female patients are 105 (75%) and male patients are 34 (35%). More number of cases are present in the age group of 45-54 i.e. 38 cases (27.33%). More number of cases is seen in females. Higher incidence of the gallstone in females as compared to males in the respective age groups. Hormonal factors resulting in biliary stasis are the main cause for increased gallstone disease in females. Pain in the gall bladder region was the most common symptom in the patients included in this study (97.3%). The second commonest symptom was fatty-food intolerance, which was present in 61.3%.reporting of the symptoms in both sexes was almost identical.

Ultrasound abdomen shows cholelithiasis in 97 cases (70%) including 44 cases(45.36%) in males and 53 cases (54.63%) in females. In 139 cases 130 cases underwent laparoscopic cholecystectomy and 9 cases underwent open cholecystectomy. Varieties of microorganisms were isolated from the bile in 24 (17.3%) out of 139 patients undergoing cholecystectomy, Common organism isolated in bile culture was E.Coli (13 cases). Our series of 139 patients consisted of 105 (75.33%) females and 34 (24.67%) males with a Female-to-male ratio of 3:1. The age pattern for patients presenting for cholecystectomy showed that most of the Patients (79; 52.67%) were belonging to the 3rd and 4th decades of life.

Table 1: AGE DISTRIBUTION (N=139)

Age groups(years)	Number of patients	percentage
15-24	4	2.87%
25-34	24	17.3%
35-44	35	25.3%
45-54	37	27%
55-64	19	14%
65-74	16	11.3%
More than 75 years	4	2.87%

Diverse varieties of microorganisms were isolated from the bile in 24 (17.3%) out of 139 Patients undergoing cholecystectomy. Aerobes were identified in all the culture-positive Patients, whereas anaerobes were not identified in any patient. Among culture-positive patients (24), Escherichia coli was the most common aerobe Identified in 13 patients (53.84%). The other frequently encountered aerobes were Pseudomonas aeruginosa (26.92%) and Staphylococcus aureus (19.23%).Sensitivity to third- and fourth-generation cephalosporins was higher as compared to second-generation cephalosporins in acute as well as Chronic cholecystitis. The antibiotics to which most of the organisms were found sensitive were cefoperazone (73.0%) and cefepime (69.23%), and almost all the organisms were resistant to cefuroxime (96.15%). In our study bile culture was positive in 24 cases out of 139

cholelithiasis cases. In R.G. Willis and W.C. Lawson series 9 cases were positive for bile culture out of 76 cases. More cases are found in the age group of 41-50 years in our study and 51-60 years in their study.

Table 2:

	Cholelithiasis	C/S cases	Percentage
	Cases		
Liggia Stewart and McLead Griffis series	215	48	22.3%
Our series	139	24	17.26%

In our study 24 cases are bile culture positive. In Liggia Stewart and McLead Griffis study 48 cases shows positivity in 215 cases.

Discussion

The Greek physician Trallianus described calculi within the hepatic radicals of human liver. 16th century – Vesalius and Fallopus described gallstones in the gall bladder Of dissected human bodies.¹⁵ In 1882 – Langenbuch widened the understanding of gall stone pathology by performing the first cholecystectomy.²¹ In 1982 Gracier and Ransohoff followed 123 Michigan university faculty members (110 men and 13 women) for 15 years who had been found to have gallstones through routine screening. At 5, 10 and 15 years follow up 10%, 15% and 18% had become symptomatic. None of them developed complications before the onset of more typical symptoms. Approximate rate at which patients developed biliary pain was 2%. Three patients developed biliary complications preceded by biliary pain.²² Atilln and colleagues also followed over 10 years 151 subjects identified to have gallstones during the GREPCO stage of subjects 33 had symptoms and 118 are asymptomatic during the study in the beginning. The cumulative probability of developing biliary colic was 12% at 2 years, 26% after 10 years. The cumulative probability of developing complications after 10 years was 3% in the asymptomatic patents and 7% in the symptomatic group.²⁰ The authors concluded that the natural history of gall stone disease might not be as benign as previously thought. Wada and Imamura (1993) found one third of 1850 patients with cholelithiasis to be symptomatic. 20% of the remaining 680 asymptomatic patients become symptomatic over a median follow up of 13 years. Patients with age more than 70 are likely to become symptomatic than age below 70 years.²³ 1983 – Gilat and co-workers studied the familial tendency of gall stonedisease.⁷

Incidence of stone in infected bile: In their study of Lygia Stervert and J. Macleod Griffiss with 215 patients with symptomatic gall stone disease, 48 patients shows bile culture positivity. The percentage is 22.3%. In our study of 139 cases, 24 cases are bile culture positive. The percentage is 26%.¹⁴ Bacteria in the bile produce glycocalyx which helps in bacterial adherence and precipitation of bilirubin pigment which act as nidus for gallstone formation. Incidence of gallstones in normal bile study and infected bile. In their study, Lygia Stewert and J. Macleod Griffiss with 215 patients with gall stone disease 177 patients with Gallstone disease shows no bile culture positivity and 48 cases shows bile culture Positivity. The percentage is 22.3%.¹⁴ In our study of 139 cases, 24 cases show bile culture positivity 115 cases showsno results. The percentage is 17.26%. In their study of 42 control cases Department of Surgery, studied 65 patients undergoing cholecystectomy for cholelithiasis, bile was cultured and stone

cholesterol content was measured. Gall bladder stones in 65 patients were identified as cholesterol gallstones in 46 patients (71%), pigment gallstones in 19 (29%) patients. Bile culture was positive in 4 patients in cholesterol gallstones, 7 cases in pigment gallstones. They conclude that black and brown pigment gallstones have different pathogenic mechanisms and that bacterial infection is important only in the formation of brown pigment stones.¹⁵

Conclusion

Gallstone disease is common in females and in the 3rd and 4th decade of life, In 134 cholelithiasis cases pain abdomen (right hypochondriac pain) was present in all cases, nausea and vomiting in 41 and 31 cases, fever in 28 cases, Ultrasound abdomen was the main investigation and shows cholelithiasis in 97 cases. Out of 139 cases 9 patients undergone open cholecystectomy and 130 patients underwent laparoscopic cholecystectomy, In 139 cholelithiasis cases 24 cases shows bile culture positivity including 19 female cases and 5 male cases.

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