

FREQUENCY OF INFECTED BILE IN PATIENTS OF UNCOMPLICATED GALLSTONE DISEASE

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ABSTRACT

Objective: To find out the association of bile infection with uncomplicated gallstone disease in our setup.

Study Design: A Case control study.

Place and duration of study: This study was carried out at Surgical Department Combined Military Hospital, Multan from 7th March 2007 to 15th September 2007.

Patient and Methods: A total of 100 patients who underwent cholecystectomy for uncomplicated symptomatic cholelithiasis and 40 controls who underwent laparotomy and had no gallbladder stone shown by ultrasound scan, were included. Symptoms, clinical signs, findings of investigations, details of treatment and postoperative care were noted. Main focus of the study was aspiration of bile for culture peroperatively. The bile was sent for culture to assess microflora and their antibiotic susceptibility. The procedures were undertaken under sterile conditions.

Results: Out of 100, 30 bile culture positive cases for different micro-organisms were detected. Escherichia coli was found to be the commonest organism in our study (40%). Next most common organism isolated was Klebsiella pneumoniae (23%). There were no bacterial isolates in the bile of controls.

Conclusion: The study demonstrated that patients with gallstone disease exhibit bactibilia in contrary to patients without gallstones who did not have bacteria in their bile, showing an association between gallstone formation and the presence of bacteria in bile.

Keywords: Symptomatic gall stone disease, Bile infection, Bactibilia.

INTRODUCTION

Cholecystectomy for either recurrent biliary colic or acute cholecystitis is the most common major surgical procedure performed by general surgeons, resulting in approximately 500,000 operations annually¹.

Gallstones may be caused by a combination of factors, including inherited body chemistry, weight, the motility of the gallbladder (the gallbladder contracts less than normal), and perhaps diet. They tend to develop in people who have liver cirrhosis, biliary tract infections and hereditary blood diseases such as sickle-cell anemia. Secondary bacterial infection with enteric organisms (most commonly Escherichia coli, Klebsiella pneumoniae, and Streptococcus faecalis) occurs in about 20% of cases²⁻⁴. It is well known that bactibilia is a common finding in high-risk individuals with complicated gallstone disease, including those with biliary

obstruction, age > 70 years, acute cholecystitis, common bile duct stones, cholangitis and non-functioning gallbladders^{5,6}. However, there are relatively few data on the prevalence of bactibilia in patients undergoing cholecystectomy for simple cholelithiasis. Reports are conflicting over the use of bile culture at surgery and the association of positive bile cultures with surgical infection and septic complications^{7,8}. Early reports showed a relationship between positive bile culture, wound infection, and other serious septic complications⁹⁻¹¹. Hence they are valuable for planning of antibiotic prophylaxis and treatment¹². Gallbladder bile from patients with uncomplicated cholelithiasis regarding different types of gallstones is rarely studied.

In developing countries like Pakistan, biliary infection is a potential etiologic factor in calculous cholecystitis^{13,14} and pre-emptive antibiotic cover in high risk suspected cases may prevent development of complications. This study was conducted to find out the frequency of infected bile and type of

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microorganisms involved, among patients of symptomatic gallstone disease.

MATERIALS AND METHODS

The case control study was conducted at combined military hospital, Multan surgical department from 7th March, 2007 to 15th Sep, 2007 and patients were sampled through non-probability convenient sampling, through out patient department.

Our inclusion criteria was first 100 patients who underwent cholecystectomy for uncomplicated symptomatic gallstone disease and 40 control patients who underwent laparotomy for large bowel cancer (15 patients), pelvic mass 15 and gastric cancer 10. During the operation, bile was aspirated from the gallbladder. The control patients showed normal gallbladder and extra-hepatic biliary anatomy on ultrasonography of them. All patients consented for research work.

Patients unwilling to comply with study protocol or immunosuppressed patients with serious co-morbid conditions and patients with high risk for bactibilia (acute cholecystitis, common duct stones, chronic liver disease, emergency surgery, and therapeutic interventions) were excluded from study. None of the patient with complicated gallstone disease or symptomless (silent) gallstones was included in this study. Abdominal ultrasound was the main tool for pre-operative diagnosis and upper gastrointestinal endoscopy was requested in few cases (where symptoms mimicked with acid peptic disease) to rule out the concomitant presence of acid peptic disease.

The operations were planned electively and the patients were admitted on the night before operation and the preoperative assessments were reviewed again. An informed consent was obtained from all patients, at which time the nature of procedure and the potential risks and related complications of cholecystectomy were explained. Perioperative antibiotic cover was provided with injection of Cefuroxime 1.5 Gram administered at the induction of general anaesthesia.

Open cholecystectomy was performed using a right subcostal incision and laparotomy by midline incision in majority of cases.

At time of surgery, bile was collected from gall bladder through 10cc disposable syringe using all aseptic measures and placed in a sterilized container. It was labeled and sent to laboratory for culture and sensitivity.

Gall bladder was removed and opened. Gallstones were classified according to visual appearance or color. The stones were separated into two groups: (1) Black stones and both black and brown stones were regarded as pigment stones, and (2) yellow stones were regarded as cholesterol stones. Biochemical analysis of stones was not carried out.

About 3ml of bile sample was inoculated into the bile broth, blood agar, MacConkey's agar and direct inoculates of culture media and plates were incubated at 37°C under aerobic conditions. Direct gram staining was done to test for the presence of organisms and pus cells. The culture plates were examined at 24 and 48 hours for bacterial growth. Bacterial growth was identified by gram staining, culture characteristics, and biochemical test through api (analytical profile index) 10 S. Then antibiogram pattern of the isolates carried out using standard technique.

Follow-up examination was performed on seventh postoperative day with bile culture/sensitivity reports.

All the data for each case, regarding each bile culture/sensitivity report was recorded on a printed proforma, as per annexure B. The age, sex and clinical features of the patients and the relevant information were also recorded. The data collected were analyzed statistically by using a computer software program SPSS 11.0 for windows.

RESULTS

In our study, 100 patients were included over the period of 6 months who underwent cholecystectomy for symptomatic uncomplicated gallstone disease and 40 patients (as control) who underwent laparotomy for diseases other than gallbladder like large bowel cancer (15 patients), pelvic mass (15) and gastric cancer (10), at CMH Multan.

The age ranged from 21 years to 84 years. The mean age was 47 years with SD±14.6.

Female to male ratio was 3.5:1 (Female - 78% and males - 22%).

Bile was collected peroperatively and sent for microbiology. Out of 100 patients (cholesterol stones - 68, pigment stones - 32), 30 cases were found positive for bile infection (Table 1). Twelve (18%) isolates were from cholesterol stone-containing bile and 18 (56%) were from pigment stone-containing bile (P=0.01, Student's t-test). Different group of micro-organisms were detected as shown in Table 2. Similarly, Samples of bile were obtained from 40 control subjects (24 men, 16 women; aged 33-70 years with a median age of 38 years). No bile samples from the controls showed bacterial growth.

Abdominal pain, varying in intensity from mild to severe, was the major complaint in 90% of patients. The pain while most frequently located in the right upper quadrant was sometimes noted in the epigastrium and occasionally in the left upper quadrant. Nausea and/or vomiting were present in 8 patients. The average duration between onset of symptoms and presentation to surgeon was relatively late (6-24 months) and mean duration of disease was 2.3 yrs with SD±3.0. Four patients underwent upper gastrointestinal endoscopy before surgery to rule out associated acid peptic disease and found to have normal study.

There was no mortality in the series. Four patients (8%) developed minor wound infection, which was managed by simple antiseptic dressings. Luckily, none of the patient developed jaundice post-operatively.

Table-1: Bile culture results (n=100)

Bile culture	No of Patients	Percentage
Positive	30	30%
Negative	70	70%

Table-2: Micro-organisms involved in bile infection (n=30).

Micro-organisms	No of Patients	Percentage
E.Coli	12	40%
Klebsiella	7	23%
Protius	4	13%
Pseudomonas	3	10%
Staphylococci	2	7%
Mixed Growth	2	7%

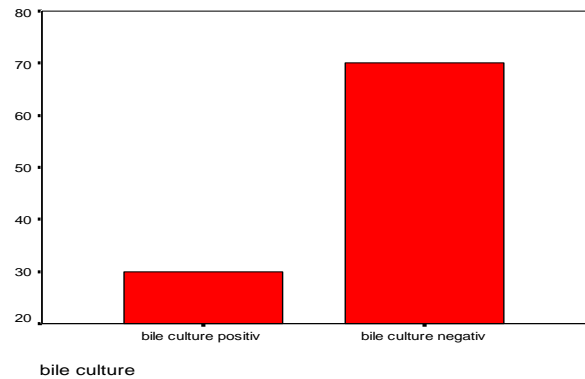


Fig 1: Bile infection frequency

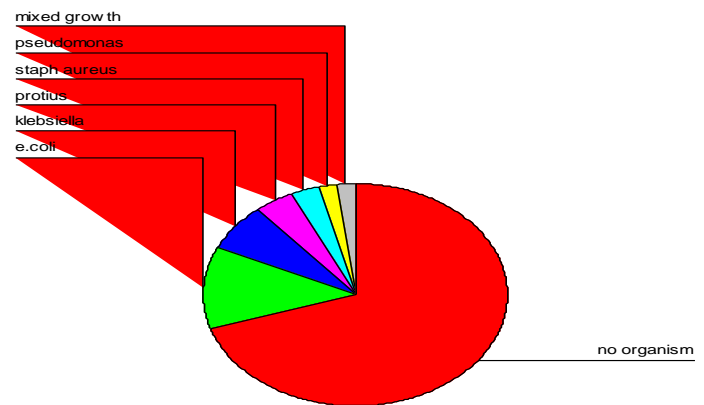


Fig 2: Micro-organisms involved in bile infection

DISCUSSION

Usually 85% of patients with gallstones remain asymptomatic for over 10 years¹⁵. About 15% of patients with gallstones are subjected to surgery for uncomplicated symptomatic gallstones¹⁶. There is a higher incidence of postoperative morbidity and infectious complications in patients with pathogenic bacteria in gallbladder bile than in patients with no bacterial growth or opportunistic bacteria¹⁷. A bacterial cause of gall stone formation has been proposed¹⁸ and positive bile cultures have been noted in 23 to 60% of patients with acute cholecystitis in previous studies¹⁹. The predominant microorganisms isolated from the gallbladder bile in these patients are Escherichia coli (60%), Klebsiella pneumoniae (22%) and streptococcus faecalis (18%)²⁰. Similarly, several

other studies have documented the nature of biliary organisms and defined infection rates in patients with gallstone disease²¹. In contrast, relatively few studies have looked at bile from patients who do not have evidence of gallstones. A study by Csendes et al²² compared the prevalence of bactibilia in normal controls (gastric ulcer surgery) to patients undergoing cholecystectomy for acute and chronic cholecystitis. They found that all controls had sterile bile while those with acute and chronic cholecystitis had positive cultures in 47% and 30% of cases, respectively.

Interest has continued to abound in the role of infection in cholelithiasis. Two fallacies, however, exist in this regard. (a) Firstly, the culture of the organism from the bile at the time of the operation does not necessarily indicate a cause-effect relationship between the infective microorganism and lithogenesis, as infection may be secondary to calculus formation. (b) Secondly, the failure to isolate organism from bile also does not indicate that the etiology is unrelated to the infection as it is well known that organism which have initiated the stone precipitation may not persist in the viable form in the bile till surgery²³.

In our study, ultrasonography was used as diagnostic tool as it has 90-95% sensitivity for gallstones. The diagnostic challenge posed by gallstones is to verify that abdominal pain is caused by stones and not by some other condition. Ultrasound or other imaging techniques can usually detect gallstones. Nevertheless, because gallstones are common and most cause no symptoms, simply finding stones does not necessarily explain a patient's pain. So ultrasonographic findings were correlated clinically to reach final pre-operative diagnosis. Characteristically gallstone pain develops suddenly in the center of the upper abdomen and spread to the right upper back or shoulder blade area. It is usually hard to get comfortable; moving around does not make the pain go away and lasts for 15 minutes to 24 hours. In patients with abdominal pain, causes other than gallstones are usually responsible if the pain lasts less than 15 minutes, frequently

comes and goes, or is not severe enough to limit activities.

The schema for classifying gallstone disease may be based on stone composition, location, and etiology. Cholesterol or mixed cholesterol and pigment stones are the most common type of stones found in the United States, and account for approximately 70% of all stones in the United States and most Western nations. Bacterial isolates were significantly more common in pigment stone-containing bile than in cholesterol stone-containing bile in our study.

Main focus of this study was aspiration of bile for culture peroperatively. A Gram stain showing 1 or more bacteria and 1 or more leukocytes and positive bile cultures suggest the diagnosis. A specificity of 87% has been reported for Gram stains and bile cultures, but the sensitivity is low.

The primary finding of the study was 30% culture positive cases for different microorganisms in patients of uncomplicated symptomatic cholelithiasis as compared to 40 control subjects without gallstone disease showing no bile colonization.

Escherichia coli was found to be the commonest organism in our study (40%) as already been reported elsewhere²⁴. Next most common organism isolated was *Klebsiella pneumoniae* (23%). However, *Pseudomonas aeruginosa* has been reported as the predominant flora by Dhir et al²⁴. Our study results are consistent with a recent study conducted by Sattar et al in which 36 out of 100 patients had positive bile culture²⁵. The most common organism was *Escherichia coli* (17 patients) followed by *Klebsiella pneumoniae* (9), *Pseudomonas aeruginosa* (6), *Staphylococcus aureus* (2), *Salmonella* (1) and *Bacteroids fragalis* (1) patient.

It may be argued that our administration of antibiotics may have adversely biased the positive culture rates of the bile. However, as the average cholecystectomy lasted little over 60 min, then it is unlikely that a single prophylactic dose of antibiotics would be efficacious against all bacteria present within the bile during this time frame.

The statistical significance of the incidence of positive and negative cultures from the patients and controls were compared using the paired t test. Significance was assigned a P value of <0.05.

CONCLUSION

The results of this study depict a clear association between bile infection and pathogenesis of gallstones as patients without gallstones did not have bacteria in their bile.

In case of cholecystitis and cholelithiasis, antibiotics can be started subsequently if the clinical situation or the culture reports warrant. Routine culture of all bile samples is, however mandatory. It is concluded that bile infection is a potential risk factor in gall stones formation.

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