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Original Research Article

Microflora of gall bladder bile in patients undergoing laparoscopic cholecystectomy

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ABSTRACT

Background: Gallstone disease is one of the most common problem affecting the digestive tract. The conditions that predispose to the development of gallstones are obesity, pregnancy, dietary factors, Crohn's disease, gastric surgery, terminal resection, hereditary spherocytosis, sickle cell disease, and thalassemia. The incidence is four times higher in women than in men with high prevalence among the age group of 30-40 years. Bacteria are commonly found in inflamed gallbladder and in patients with cholelithiasis, whereas evidence suggests that normal bile is sterile. So, exploring and identifying the microflora of gallbladder bile helps to choose appropriate antibiotics.
Methods: Minimum of 50 Patients admitted under General Surgery, who are scheduled to undergo laparoscopic cholecystectomy. Five ml of bile will be aspirated from the gall bladder during laparoscopic cholecystectomy. The samples will be transferred onto a plain sterile container and then transferred at room temperature in an hour to the laboratory for culture.
Results: In the present study 50 patients were evaluated for culture and gall bladder bile. Out of which maximum number of patients were clustered between 31-40 years. In the present study out of 50 patients, 34 (68%) were females and 16 (32%) were males. In the present study We found total of 33 (66%) yellow stones, 13 (26%) were black in colour and 4(8%) were brown stones. In this study bile culture was showing growth of the following organisms. The commonest organism was E. coli (64%) followed by Klebsiella (25%) and Enterococcus (11%).
Conclusions: Bactibilia has long been known to be associated with biliary tract diseases and culturable bacteria in bile can represent a state of asymptomatic bactibilia which can disseminate after any intervention causing infective complication. Exploring the microflora of gall bladder bile important role in choosing the appropriate antibiotic to prevent complications.

Keywords: Cholesystecty, Gall bladder, Microflora

INTRODUCTION

Gallstone disease is one of the most common problem affecting the digestive tract. The conditions that predispose to the development of gallstones are obesity, pregnancy, dietary factors, Crohn's disease, gastric surgery, terminal resection, hereditary spherocytosis, sickle cell disease, and thalassemia. The incidence is four times higher in women than in men with high prevalence among the age group of 30-40 years.[1]

It is a benign disease of the biliary tract is one of the most common indications for major abdominal surgery in India. Bactibilia has long been known to be associated with biliary tract diseases and culturable bacteria in bile can represent a state of asymptomatic bactibilia which

can disseminate after any intervention causing infective complication.[2]

The presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization of the bile. In patients without stone disease, previous biliary intervention is associated with high rates of bacteriobilia. Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Upon bile duct obstruction, bacteria proliferate within the stagnant bile while biliary pressure increases. Eventually, the bacteria presumably translocate into the circulation causing a systemic infection. Acute cholangitis spans a continuous clinical spectrum and can progress from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome. Acute cholecystitis is an infection of the biliary tract, which results from bile stasis due to chronic obstruction. The obstruction is usually attributed to gallstones in 80% of cases. The causes of acalculous cholecystitis include biliary strictures, human immunodeficiency virus cholangiopathy, biliary parasites and primary sclerosing cholangitis. Other causes include complicated cases of burns, trauma, major surgery, diabetes and unusual bacterial infections of the gallbladder (*Salmonella* spp. or *Vibrio cholerae*) and other systemic infections (tuberculosis and syphilis).[3]

Bacterial infection has been considered a primary factor, not only in the pathogenesis of brown stones, but also in the formation of black pigment stones. Although bile is usually sterile, bacteria isolated from intraoperative bile culture was 25%, 66%, 67% and 9% for gall bladder (GB) stones, common bile duct (CBD) stones, intrahepatic duct (IHD) stones and biliary malignancy, respectively. The bacteria isolated from bile in all disease states of the biliary tract are primarily gram-negative enteric coliforms. Escherichia coli (E. coli) alone or mixed with another organism is present in 50% of the positive cultures.[4] Other coliforms e.g. Klebsiella, Enterobacter and proteus are less commonly isolated. Streptococcus faecalis (enterococcus) is an aerobic gram-positive coccus that is also frequently isolated. Anaerobic microorganisms are isolated in less than 10% of the cases, clostridium perfringens being the most common.[4] Aerobic organisms cause 94% of biliary tract infections while anaerobic organisms cause the rest. Bacteria are commonly found in inflamed gallbladder and in patients with cholelithiasis, whereas evidence suggests that normal bile is sterile. Inflamed gallbladder has markedly altered permeability, which permits absorption of bile acids and movement of inorganic salts into the gallbladder lumen. The role of excessive cellular debris and increased protein secretion, which occurs in response to inflammation, may be present. Finally, bacterial enzymes effects constituency of bile which may alter its solubility leading to precipitation of bile salts. Most gall stones are composite in nature. Bacteria can be found in most pure stone (i.e., those whose structure consists more than 90% cholesterol).[5]

Laparoscopic cholecystectomy is now the treatment of choice for symptomatic gallstone disease. Elective laparoscopic cholecystectomy (LC) has a low risk for infective complications. The incidence of surgical infections after LC is reported to be less than 2%, because of the minimal trauma due to this approach.[4]

METHODS

It was a cross sectional study carried out in the Department of Surgery, MVJ Medical College for 5 months January to May 2018. Minimum of 50 Patients admitted under General Surgery, who are scheduled to undergo laparoscopic cholecystectomy and willing to give the consent, will be included in the study if they fulfil inclusion criteria. It was a simple random sampling method.

Five ml of bile will be aspirated from the gall bladder during laparoscopic cholecystectomy through 10 mm port by using long aspirating needle, while the gall bladder is in situ, in all the selected patients. The samples will be transferred onto a plain sterile container and then transferred at room temperature in an hour to the laboratory for culture.

Inclusion criteria

- Patients with chronic calculus cholecystitis.
- Patients with uncomplicated symptomatic cholelithiasis.

Exclusion criteria

- Patients who receive antibiotics one week before operation.
- Patients with diabetes mellitus.
- Patients on regular corticosteroids.
- Patients with elevated liver enzymes twice the reference level.
- Patients with acute cholecystitis.
- Patients not willing to participate in the study.

Study variables

- Age (years)
- Sex
- Address

Patients will be informed regarding the aims and objects of the study in their local language and a detailed informed written consent will be taken from the patient (in case of minor less than 18 years consent from the parent will be taken and assent from the patient will be taken) prior to inclusion into the study (Appendix II).

During hospitalization relevant history will be collected and appropriate investigations as deemed necessary will be done using standard procedures.

RESULTS

In the present study 50 patients were evaluated for culture and gall bladder bile. Out of which maximum number of patients were clustered between 31-40 years of age (44 %), followed by 41-50 years of age (26%) and remaining patients were in the age group of 51-60 years (6%).

Table 1: Number of patients according to age distribution.

Age	Number	Percentage
21-30	12	24
31-40	22	44
41-50	13	26
51-60	3	6
Total	50	100

Present study showed highest percentage of people in age group 31-40. Three patients belonged to group age 51-60 which contributed to 6 percent.

In the present study out of 50 patients, 34 (68%) were females and 16 (32%) were males.

Table 2: Sex distribution.

Sex	Number	Percentage
Male	16	32
Female	34	68

In the present study authors found total of 33 (66%) yellow stones, 13 (26%) were black in colour and 4 (8%) were brown stones.

Table 3: Distribution of different colour stones.

Colour	Number	Percentage
Yellow	33	66
Brown	4	8
Black	13	26
Total	50	100

In this study out of 50 bile samples, 13 (26%) were culture positive and 37 (74%) were culture negative. There were more number of culture negative than culture positive.

Table 4: Percentage of bile culture.

Culture	Number	Percentage
Positive	13	26
Negative	37	74
Total	50	100

In this study bile culture was showing growth of the following organisms. The commonest organism was E. coli (64%) followed by three had Klebsiella which contributed to (25%) and Enterococcus (11%) which was seen in two patients.

Table 5: Percentage of the growth of organisms.

Organism	Number	Percentage
E. coli	8	64
Klebsiella	3	25
Enterococcus	2	11

Bile was aspirated from different sites of gall bladder. 20 samples were taken from body, 18 samples from fundus and from neck 12 samples.

Table 6: Frequency of aspiration site and growth of organisms.

Frequency		Organism		
		E. coli	Klebsiella	Enterococcus
Body	20	3	1	-
Fundus	18	1	0	1
Neck	12	4	2	1
Total	50	7	3	2

E. coli had more predominance to neck .18 cases of aspiration in fundus revealed one E. coli, and one Entrerococcus.20 aspirates from body had revealed E coli in three aspirates Klebsiella in one aspirate.

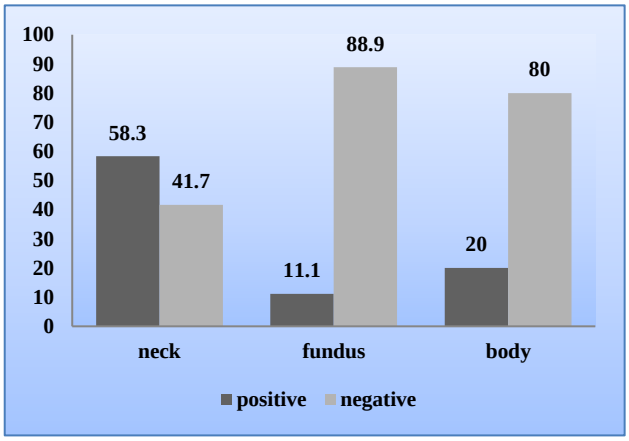

Figure 1: Frequency of aspiration.

Post-operative stays in majority of patients (37/50) was median of 2 days.

Table 7: Number of days of the hospital stay of the patients.

Days	Number of patients	Percentage
1	2	4
2	37	74
3	11	22

More number of patients around 37 patients contributing to 74 percentage had hospital stay around 2 days. Two

patients had hospital stay around one day. 22 percent of patients amounting to 11 had hospital stay around 3 days.

DISCUSSION

Age and sex prevalence

In the present study culture and sensitivity of gallbladder bile in 50 patients undergoing laparoscopic cholecystectomy were evaluated.

Among them maximum number of patients were clustered between 31-40 years of age (44 %) and 41-50 years of age (26%) and females were 34 (68%) and males were 16 (32%). The female and male ratio is 2.1:1.

Nagaraj SK et al in their study found the highest incidence was seen in patients in the age group of 41-50 years. The female to male ratio was 2.57:1. Pradhan SB et al have done a study to see the prevalence of different types of gallstone in Nepal and to correlate them with the clinical findings. The most commonly involved age group for cholelithiasis (32.5%) is found to be 30-39 years with a female predominance (M: F=1:3.2).[6]

Taher MA et al did a prospective study in 75 patients with gallstone (66 females and 9 males). The ratio of females: males was (7.3:1). Sharma R et al in their study gallstones were found more frequently in women than men. The ratio of male to female gallstone patients was about 1: 3. It was seen that 72.6% of the cases were in the age of 21-50.[7]

Rakesh BH et al found that the highest age incidence of chronic calculous cholecystitis was in the 5th decade, more common in females, majority with pain abdomen.[8]

Mohan et al also found gallstones with preponderance of female (M: F ratio 1:6.4).

The result of present study showed female preponderance of 2.1:1 and maximum number of patients were clustered between 31-50 years of age (70 %) and comparable with Nagaraj SK et al, Pradhan SB et al and Sharma R et al, other studies done by Mohan et al and Taher M A were shown highest preponderance of females than present study.[9]

Bile culture

In present study out of 50 patients undergoing laparoscopic cholecystectomy 13 were positive (26%) and 37 were sterile (74%). The commonest organism was E. coli 16 % (8 out of 13) and other organisms were Klebsiella 6% (3 out of 13) and Enterococcus 4 % (2 out of 13).

Chang WT et al in their retrospective study on bacteriology in biliary tract disease on 1394 patients including gallbladder (GB) stones, common bile duct (CBD) stones, intrahepatic duct (IHD) stones, GB polyps and biliary malignancy found the overall positive rate of bile culture is 36% in their study while it was 25%, 66%, 67% and 9% for GB stones, CBD stones, IHD stones and biliary malignancy, respectively.[4] For gallstone diseases, the most common organisms cultured were Gram negative bacteria (74%), in which Escherichia coli (36%) and Klebsiella (15%) were most commonly found, followed by Gram positive (15%) bacteria such as Enterococcus (6%) . Another study by Harbi M et al on 112 patients undergoing elective laparoscopic cholecystectomy for gallstones. Of 112 bile samples examined, 28 (25%) were culture positive, four of which contained more than one organism. The most common organisms isolated were Escherichia coli 9 (28.1%), Enterococcus faecalis 5 (15.6%) and Pseudomonas aeruginosa 3 (9.4%). In one sample they found Aeromonas hydrophilia and Enterobacter cloacae. No anaerobes were detected.[10]

Morris GJ et al found that the overall prevalence of viable bacteria within bile sampled at cholecystectomy was 15.6%. Khan AB et al in their study on 121 patients found 42/121 (34%) patients were bile culture positive while 79/121 (66 %) patients were bile culture negative. Pushpalata H et al also found Bactibilia in 27/50 (52%) of patients. Polymicrobial flora was found in 10% of bile samples. *E. coli*, *Klebsiella pneumonia* and *Enterococcus faecalis* were the predominant organisms isolated. Sattar I et al found common infecting organisms in 100 cases of cholelithiasis.[1] Out of 100, 36 patients had positive bile culture. The most common organism was E. coli (17 patients) followed by Klebsiella (9), Pseudomonas (6), Staphylococcus aureus (2), Salmonella (1) and Bacteroids fragalis (1) patient. Ozturk A et al in their study on 112 patients bacterial growth was detected in the bile culture of 15 patients (13.1%). The most commonly isolated bacteria were Enterococcus sp (4 patients, %26.6%), Escherishia coli (3 patients, 20%) and Enterobacter sp (3 patients, 20%). Eslamil G et al study shown 63 samples bacterial growth out of 100 bile samples, of which 11 revealed two species of bacteria. A total of 10-gram positive (enterococci and staphylococci) and 53 gram negative (E. coli, Klebsiella, Aerobacter, Pseudomonas, Proteus, Citrobacter, Providencia And Acinetobacter) bacteria were isolated. Malik ZI et al found out of 100, 32 patients had positive bile culture from gallbladder. The most common organism was *Escherichia coli* (14 patients) followed by Proteus (8), Pseudomonas (4), *Staphylococcus aureus* (2), Streptococcus (2) and Anaerobes (2).

Mozafar M et al in their study found 47 cultures positive out of 130 bile culture results (36%). *Escherichia coli*, Klebsiella and *Staphylococcus aureus* were the most common cultured bacteria.

In present study total culture positive was 26 % it is comparable with studies done by Harbi et al, Khan AB et al, Sattar I et al, Malik ZI and Mozafar M et al.

Pushpalata H et al found bactibilia of 52% which was more than present study of 26 %. But Ozturk A et al found only 13.1% less than present study of 26 %.

Colour of the stones and its relation with bile culture

In present study authors found 33 yellow colour stones, 13 black colour stones and 4 brown colour stones. In that 21.2% of yellow stones, 30.8% of black stones and 50% of brown stones were culture positive. More common type of stone was yellow colour stone 66 % followed by black 26 % and brown 8 %.

Kaufman HS et al in their study on gallbladder stones in 65 patients were identified as cholesterol in 46 (71%), black pigment in 17 (26%), and brown pigment in 2 patients (3%). Bile cultures were positive in 13% of patients with cholesterol stones, in 14% of those with black pigment stones, and in all of the patients with brown pigment stones.

Pradhan SB et al was also found mixed stone to be the most common type of stone comprising 78.75%, followed by cholesterol stone 12.5%, brown pigment stone 7.5% and black pigment stone 1.25%.

Rakesh BH et al found 60% of the pigment type gallstones in 50 cases of chronic calculous cholecystitis.

Taher MA et al found cholesterol stone was the most prevalent type of stones, the percentage was (49.3%) greater than mixed and pigment stone where the percentages were (33.3%) and (17.3%) respectively.

Mohan et al on morphological analysis found gallstones were of mixed type in 686 cases (62.3%), pigment stones in 34 cases (3.2%), cholesterol type in 182 cases (17.3 %) and combined type in 148 cases (14%).

The prevalence of different type of stones in present study found that yellow was commonest, followed by black then brown, which is comparable with studies done by Kaufmann HS et al, Pradhan SB et al and Taher MA et al but Rakesh BH et al found highest prevalence of pigment type stones. Culture growth of organisms was found high in brown colour stones (50%) followed by 30.8% in black stones and 21.2% in yellow stones, which is comparable with other studies.

Site of aspiration and culture positivity

In present study bile was aspirated from different parts of the gallbladder. Body (20), Fundus (18) and Neck (12). The culture positivity with respect to site of aspiration was Body 20 % (4/20), Fundus 11% (2/18) and Neck 59% (7/12).

Manolis EN et al in their cohort study thirty-one patients (22.6%) demonstrated at least one positive culture sample. Positivity was 31/31 (100.0%) in neck samples, 20/31 (64.5%) in body and 13/31 (41.9%) in fundus samples (P<0.001).

Present study shows that neck samples were more likely to be positive than other parts of the gallbladder and in comparison, to Manolis EN et al study also shows that the neck of the gallbladder hosts the biggest bacterial load in comparison with the body and the fundus.

CONCLUSION

Gallstone disease is one of the most common problem affecting the digestive tract. Bactibilia has long been known to be associated with biliary tract diseases and culturable bacteria in bile can represent a state of asymptomatic bactibilia which can disseminate after any intervention causing infective complication. The most prevalent complication of gallstone is chronic cholecystitis occurs in approximately 4% of cases. Exploring the microflora of gall bladder bile important role in choosing the appropriate antibiotic to prevent complications.

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