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Original Research Article

Morphological spectrum of gallstone and bacteriology of bile in patient of cholelithiasis visiting tertiary care centre in North India

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ABSTRACT

Background: Calculus biliary tract disease continues to be a major health concern. It is normal to see predisposing factors such as cirrhosis, ileal resection in the West; while infection predominates in South East Asia as a cause. We prospectively examined gall stones in this study to investigate the causes and role of bacteria in the disease of gall stone disease.
Methods: This is a cross sectional study conducted in department of surgery, Sri Guru Ram Das University of Health Sciences, Sri Amritsar, India, from November 2018-August 2020. Total 50 cases were selected and operated by lap/open cholecystectomy were included in this study. During cholecystectomy bile was aspirated and was sent to laboratory for culture. Gallstone retrieved from the specimen was classified based on morphological appearance.
Results: Gallstone disease found to be common in female 92% and in the age group of 4-50 years. Bile of gallstones patients is often infected. In this study though morphologically cholesterol stones were commonest but mixed stones were associated with highest infection rates. Most common bile infecting bacterium in all kinds of stones was found to be *E. coli* 60.8% followed by klebsiella 17.3% and proteus 17.3%. All the organisms cultured were sensitive to cephalosporins, quinolones, aminoglycosides and penicillin group of antibiotics.
Conclusions: Bile culture was positive in 46% of the cases. Morphologically cholesterol stones were commonest 52% but mixed stones were associated with highest infection rates (80%). Most common bile infecting bacterium in all kinds of stones was found to be *E. coli*.

Keywords: Bacteriological profile, Bile culture, Gallstone

INTRODUCTION

Gallstones are most common biliary pathology and are major cause of morbidity and mortality throughout the world. Incidence of gallstone increases with age; it is more common in female than male.[1,2] Gall stones are rare in Africa with prevalence of less than 1% and in Japan it has been increased from 2% to 7%.[3] In India it is estimated to be around 3-4%.[4] The process of formation of gallstone is complex and it varies according to type of gallstone. Many factors have been implicated in the formation of gallstones including age, gender, obesity, lithogenicity, rapid weight loss, diabetes mellitus. Number of pregnancies, post-operative periods, family history, oestrogen replacement therapy, serum lipids, drugs and decreased physical activity.[5] Amongst which infection of bile is also an important factor. Studies on human and animals have proven the mechanism by which Beta-Glucuridase producing *E. coli*, *Staphylococci* and *streptococci* can cause billary stone formation. Phospholipase producing bacteria and bacterial hydrolases leading to deconjugation of bile acids leads to

stone formation.[6-12] Amount and properties of mucin secreted by infected gall bladder can lead to a nidus formation for gall stone.[13-16] Bacteria can lead to stone formation by many other pathways e.g. prostaglandin secretions, cellular inflammatory response, lipopolysaccharide productions etc.[17-29]

The present study is aimed to determine to determine the morphology of gall stones grossly in patients undergoing cholecystectomy coming to tertiary care center in North India, to find the incidence of bile culture positivity with different types of gallstones, association of types of stone with positive bacterial culture type of bacteria associated with bile stones.

The study is also aimed to answer whether the bacteria are possibly etiological agent or not. It will also help to examine the causes for and the role of bacteria in pigment gall stone disease

METHODS

This was a Cross sectional study which was conducted on 50 patients admitted in Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India, after clearance from institutional ethical committee with diagnosis of gallstone disease and admitted for open or laparoscopic Cholecystectomy.

For this cross-sectional study, from November 2018 to August 2020, all patients over 15 years of age diagnosed with gallstone disease admitted to hospital planned to undergo cholecystectomy (open/laparoscopic) were taken for this cross-sectional study.

Sample collection was done by aspiration of bile from excised gallbladder and was sent in sterile bottle in microbiological laboratory for culture. Gallstones retrieved were classified according to gross appearance (Table 1).

Table 1: Morphology of various types of stone.

Type of stone	Morphology
Cholesterol	Solitary oval large, granular surface, yellow white. Cut section- radiating glistening crystals.
Black pigment	Multiple small jet black, mulberry shaped. Cut section -soft and black.
Mixed	Multiple, multifaceted, of variable size. Cut section - alternating dark pigment layer and white layer.
Combined	Usually solitary large, smooth. Cut section - central nucleus of pure stone with mixed outer shell or vice versa.

Inclusion criteria

Patients with confirmed diagnosis and investigatory support for the diagnosis of cholelithiasis and age above 15 years.

Exclusion criteria

The patients with h/o jaundice, common bile duct stone, *acalculus cholecystitis*.

Sample collection was done by aspiration of bile from excised gallbladder and was sent in sterile bottle in microbiological laboratory for culture. Gallstones retrieved were classified according to gross appearance

Ethical clearance for this study was approved by the Institutional ethics committee

Statistical method

The data has been analyzed using SPSS 24.0 software. Chi square test has been used to evaluate and interpret the data. P value less than 0.05 are considered statistically significant

RESULTS

In our present study, 50 diagnosed cases of gallstone disease between age group of 22 to 60 years were observed. Out of 50 cases 46 were female (92%) and 4 were male (8%).

The youngest patient was 22-year-old and oldest was 60-year-old. Mean age group was 41-50 years (42.7 years) years with age ranging from 21-60 years. Maximum number of cases were observed in the age group of 41-50 i.e. 18 (36%) (Table 2).

Table 2: Age distribution of cholelithiasis cases.

Age group (years)	No. of cases	% age
≤30	6	12.0
31-40	15	30.0
41-50	18	36.0
51-60	11	22.0
Total	50	100.0

Majority of cases were seen in females 46 cases i.e. 92%. Increased serum VLDL and hormonal factors resulting in biliary stasis is the possible explanation for increased gallstone disease in females (Table 3).

In majority of cases, morphology was found to be cholesterol stones (no. 26) with female predominance (no.-25) mixed pigmented gall stones were found predominantly in females (no.-15) and less in male (no.-2). Next common morphology was black pigmented

gallstones in (no.-5) patients, amongst which 4 were female. Least common morphology was found to be combined gallstones in (no.-4) cases, all of which were female (Table 4).

Table 3: Sex distribution of cholelithiasis cases.

Sex	No. of cases	%age
Female	46	92.0
Male	4	8.0
Total	50	100.0

Table 4: Distribution of patient according to type of gallstone based on morphology.

Morphological type of gallstone	Male	Female	Total cases
Cholesterol gall stone	1	25	26
Black pigmented gallstone	1	4	5
Mixed pigmented gall stone	2	13	15
Combined gallstone	0	4	4

X^2: 2.515; df:3; p=0.472

In 50 cases of gall stones disease cholesterol stones were found in 26 cases, with 6 cases (23%) culture positive. Black pigmented gallstones were found in 5 cases out of which 2 (40%) were culture positive. Mixed (brown) pigmented stones were 15 and of those 12 (80%) were culture positive. Only 4 cases were having combined stones, with 3 cases (75%) culture positive (Table 5).

Table 5: Collective incidence and type of gallstones with bile culture positivity in different type of gallstones.

Stone	Bile culture			
	Positive		Negative	
	No.	%age	No.	%age
Cholesterol	6	23.08	20	76.92
Black pigmented	2	40.00	3	60.00
Mixed	12	80.00	3	20.00
Combined	3	75.00	1	25.00
Total	23	46.00	27	54.00

Distribution of patient according to age and bile culture report for various types of gallstone

Majority of cases with cholesterol stone were in age group 41-50 years (11) and bile culture positivity was also maximum (4 cases) (Table 6A).

In patients with black pigmented stone, majority of the patient belonged to age group of 41-50 years and bile culture was positive in 2 cases of this age group (Table 6B).

Table 6: (A) distribution of patient according to age and bile culture report for cholesterol type of gallstone.

Age (in years)	Cholesterol stone bile culture			
	Absent		Present	
	No.	%	No.	%
≤30	2	7.69	1	3.85
31-40	5	19.23	0	0.00
41-50	7	26.92	4	15.38
51-60	6	23.08	1	3.85
Total	20	76.92	6	23.08

X^2: 3.076; 3; p=0.380

Table 6: (B) distribution of patient according to age and bile culture report for black pigmented of gallstone.

Age (in years)	Black pigment stone bile culture			
	Absent		Present	
	No.	%	No.	%
≤30	1	20.00	0	0.00
31-40	1	20.00	0	0.00
41-50	0	0.00	2	40.00
51-60	1	20.00	0	0.00
Total	3	60.00	2	40.00

X^2: 5.000; 3; p=0.172

Table 6: (C) distribution of patient according to age and bile culture report for mixed pigmented of gallstone.

Age (in years)	Mixed stone bile culture			
	Absent		Present	
	No.	%	No.	%
≤30	1	6.67	0	0.00
31-40	2	13.33	7	46.67
41-50	0	0.00	3	20.00
51-60	0	0.00	2	13.33
Total	3	20.00	12	80.00

X^2: 5.278; 3; p=0.153

Table 6: (D) distribution of patient according to age and bile culture report for mixed pigmented of gallstone.

Age (in years)	Combined bile culture			
	Absent		Present	
	No.	%	No.	%
≤30	1	25.00	0	0.00
31-40	0	0.00	0	0.00
41-50	0	0.00	2	50.00
51-60	0	0.00	1	25.00
Total	1	25.00	3	75.00

X^2: 4.000; 2; p=0.135

In patients with mixed pigmented stone, majority of the patient belonged to age group of 31-40 years (9 cases)

and bile culture was positive in 7 cases in this age group (Table 6C).

In patients with combined pigmented stone majority of the patient fell in age group of 41-50 years (2 cases) and bile culture was positive in both the cases (Table 6D).

In our study out of 6 bile culture positive cholesterol gallstones *E. coli* was the most common isolated organism (5 cases) and was sensitive to aminoglycosides, cephalosporin, fluroquinolone and tigecycline. Staph. hominis was isolated from one sample which could be due to the contamination while obtaining or transportation of sample.

In black pigmented stone bile culture was positive in 2 cases and all contained Klebsiella as the main organism which was sensitive to penicillin, aminoglycosides and cephalosporin.

In mixed stone, *E. coli* was the most common isolated organism and was found to be sensitive cephalosporin, penicillin, aminoglycosides, fluroquinolone and tigecycline. Next common cultured organism from mixed stone bile was proteus which was sensitive to cephalosporin and aminoglycosides. Klebseilla was the third type of bacterium cultured which showed sensitivity to penicillin, aminoglycosides and cephalosporin.

In combined gall stones, *E. coli* was most common isolated bacterium and was sensitive to cephalosporin, aminoglycosides and tigecycline. Proteus was the next organism which was sensitive to cephalosporin and aminoglycoside.

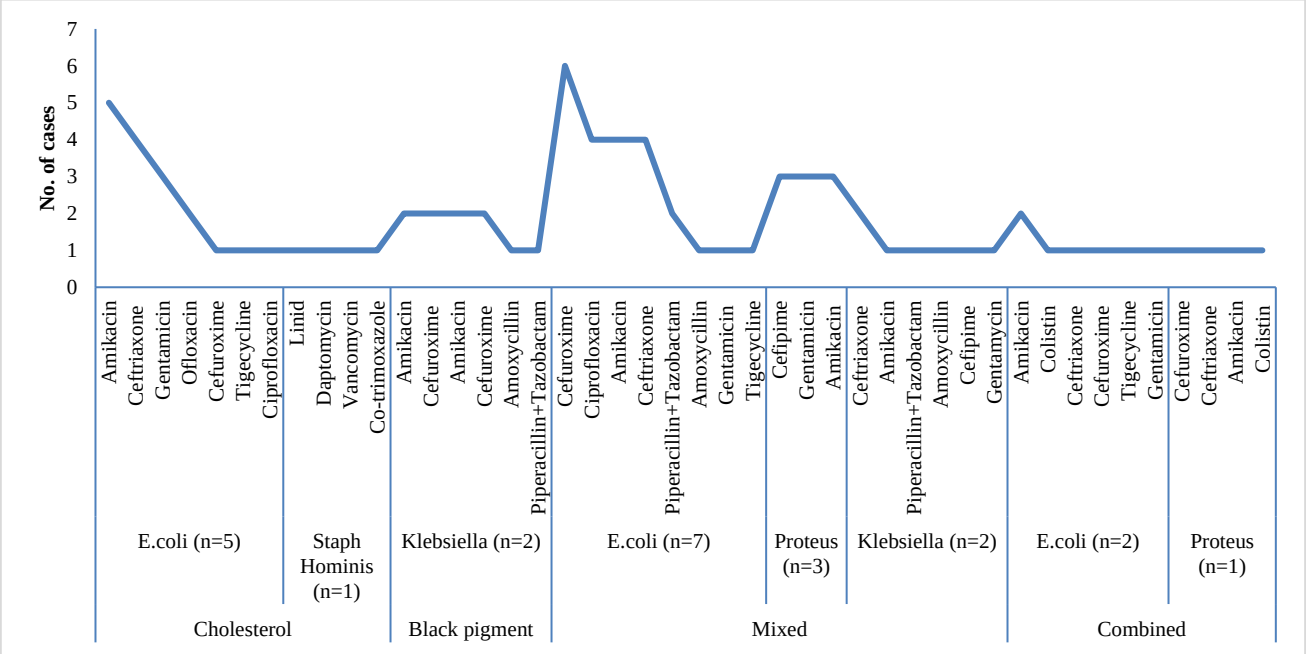

Figure 1: Type of gall stone and common microorganisms isoalted from bile culture with culture and senstivity.

DISCUSSION

In my study the majority of the patients belonged to age group 41-50 years. In a study conducted by Zuhair et al peak age of incidence was reported between 41-50 years. Study by Ranshoff reported the same results.[30-31]

Majority of patients with cholelithiasis were female. Similar female predominance was reported by Gupta et al in a study on morphological spectrum of gallstone and bacteriology of bile in cholelithiasis. Khedkar et al reported female predominance with cholelithiasis which is consistent with the study conducted.[32,34]

Gallstones were classified according to their morphological appearance and cases with cholesterol type of gallstone were found to be predominant 52%, followed by mixed stone 30%, cases with black pigment type of gallstone were 10% and cases of combined pigment stone were 8%. Some studies available in literature suggests the same incidence of gallstones morphology e.g. Tandon. Prevalence and type of biliary stones in India. World J Gastroenterology 2000; reported predominance of cholesterol gallstones among the North Indians however at the same time Gupta et al reported maximum cases with mixed type of gallstones 50%, followed by cholesterol stone 30%. Cases with combined type of gallstone were 12% and cases of pigment stone was 8% in this study.[33,34] This variation in morphology of different types of gallstones varies in different parts of India. One of the reasons can be food habits, personal hygiene etc.

In present study, the bile culture was positive in 46%, which is considerably higher than reported by Yaqin and sultan 25.7%. Guo from China showed the incidence of

bacteria to be very high ranging from 20-96% with an average of 66.7% depending on the type of gallstones and is more common in mixed type of gallstone.[35-36] Gupta et al reported the incidence about 40%.[34]

In current study, incidence of total bile culture positivity and bile culture positivity associated with particular morphology of stones is comparable with that available in previous studies. Bile culture positivity was highest in the cases of mixed type of gallstone i.e. (12/15) 80%, and the most common organism isolated was *E. coli* followed by Proteus and Klebseilla. In the study by Ohdan et al incidence of bile culture positivity was 38% and 83% in mixed type of stone and *E. coli* was the most common organism isolated.[37] Gupta, et al reported bile culture to be in positive more in the cases of mixed type of gallstone (15/25) 30%, and the most common organism isolated was *E. coli* followed by Klebseilla.[34]

E. coli was found to be the most common organism in this study. However, in the study by Sabir, Klebsiella pneumonae was reported to be most common. Attila cescend et al (52%), Willis. Stewart et al (44%), Balla et al (46%) study all reported *E. coli* to be the most common bacteria isolated.[38-40] Gupta et al also reported the most common organism to be *E. coli* followed by Klebseilla pneumonae.[34]

All of these biliary organisms were found to be sensitive to cephalosporins, quinolones, aminoglycosides and penicillin group of antibiotics specially checked for 2nd generation cephalosporin.

The importance of the predominance of *E. coli* is seen by the fact that older studies have shown glucoronidase enzymatic activity of *E. coli* to have role to play in calcium billirubinate gallstone formation.[3-13]

The infection of bile could not be established as a cause of stone formation but is associated with billary stone as shown in Table 5 and Table 6 and test for association (chi-square test) of gallstone with infection shows that we cannot exclude the possibility of them being an innocent bystander.

Limitations

The study had a small sample size and further studies with large sample size are needed. Moreover, due to design of the study there is ecological fallacy.

CONCLUSION

Bile culture was positive in 46% of the cases. Morphologically cholesterol stones were commonest 52% but mixed stones were associated with highest infection rates (80%).

Most common isolated organism from bile culture was *E. coli* 60.8% followed by klebsiella 17.3% and proteus 17.3%. All the organisms cultured were sensitive to cephalosporins, quinolones, aminoglycosides and penicillin group of antibiotics. Cholesterol stone was found to be least infected and the most common infecting organism in infected cases was *E. coli.*

From our present study we can recommend appropriate antibiotic based on the morphology of the gallstone seen during cholecystectomy and can advise appropriate antibiotic, without going for bile culture and sensitivity in each and every case.

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