Original Research Article

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Study of morphological spectrum of gallstone and bacteriology of bile in cholelithiasis

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ABSTRACT

Background: Biliary calculus disease is one of the most common disorders of gastrointestinal tract. Gall bladder stone are crystalline structure formed by concretion of bile constituents. Aetiology of gall stone is multifactorial. Bile is found to be sterile in normal patient.

Methods: This is a cross sectional study conducted in department of surgery, Lt Lakhiram Agrawal Memorial Medical College, Raigarh, Chhattisgarh, India from October 2015 - October 2016. 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 with ratio of 4:1, with maximum no. of cases found in the age group of 35 to 45 year. Bile culture as positive in 40%. The most common organism was *E. coli* isolated in 14 cases, followed by *Klebseilla* in 4 cases.

Conclusions: The frequency of bile infection in this study area was 40%, in which association with mixed type gallstone was found to be significant and the most common bacteria isolated was *E. coli*.

Keywords: Bacteriological profile, Bile culture, Gallstone

INTRODUCTION

Gallstones are a major cause of morbidity and mortality throughout the world. Gall stone are responsible are responsible for more than 95% of biliary tract disease. Incidence of gallstone increases with age; it is more common in female than male.^{1,2}

The pathogenesis of gallstone is multifactorial. It varies according to type of gallstone. Different factors have been implicated amongst which infection of bile is also an important factor. Gallstone varies in their composition majority being mixed and combined gallstone which accounts of 80% and pure gallstone contributing of 20%.³

Mixed gallstone is frequently associated with cholecystitis. Bile which in normal is sterile is found to be positive in 50% those with gallstone disease. These bacteria reach the gallbladder via bloodstream from infective focus found elsewhere in body. The biliary infection can be caused by any type ranging from aerobic gram positive to gram negative to anaerobic organisms.

Aerobic causes 94% of biliary tract infection while anaerobic causes the rest. The most common organism isolated includes *E. coli, Klebseilla,* and *Streptococcus.*⁴⁻⁶ The present study was aimed to find most common type of gallstone in this part of country which will contribute to understand its etiopathogenesis. Also to know the

frequency of bile culture positivity with its association with type of gallstone.

METHODS

This is cross sectional study which was conducted on 50 patient admitted in Lt. Lakhiram Agrawal Memorial Medical College, Raigarh, Chhattisgarh, India, with diagnosis of gallstone disease and were admitted for open

or laparoscopic Cholecystectomy from October 2015 - October 2016.

All patients above 15 year of age with the diagnosis of gallstone disease were admitted in hospital. The patients with h/o jaundice, Common bile duct stone, acalculus cholecystitis, and those who refused for surgery were excluded. Detail history of every patient was taken and complete physical examination was done.

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

Ultrasonography was the main diagnostic tool. Patient who did not have gallstone on ultrasonography were excluded. Sample collection bile aspirated from excised gallbladder and was sent in sterile bottle in microbiological laboratory for culture. Gallstone retrieved were classified according to morphological appearance.

RESULTS

In the current study, gallstone disease was more common in females (Male: Female ratio was 1:4). The age of patient varied from 21-70 years, and maximum number of cases were found between 35 to 45 years. On the basis of morphological analysis it was found that maximum percentage of cases had mixed type of gallstone (50%) followed by cholesterol (30%), combined (12%) and pigment type (8%).

Table 2: Age and sex wise distribution of study subjects.

Age group	Age group No. of cases		Total	Donoontogo
in years	Male	Female	cases	Percentage
21-30	1	5	6	12%
31-40	2	10	12	24%
41-50	2	13	15	30%
51-60	4	5	9	18%
61-70	1	7	8	16%
Total	10	40	50	100%

Bile culture was positive in 40 % of cases. The association was found significant between type of stone and bile culture positivity. In this study incidence of bile culture positivity was found more in cases with mixed

type (15/25) of gallstone and combined stone (3/6) rather than Cholesterol and Black pigment gallstone. Common microorganism isolated from bile was *E.coli* followed by *Klebseilla*.

Table 3: Distrubition of patient according to type of gallstone based on morphology.

Type of colletone	No. of cases		Total
Type of gallstone	Male	Female	
Cholesterol	4	11	15
Black pigment	1	3	4
Mixed	5	20	25
Combined	0	6	6
Total	10	40	50

Table 4: Frequency of positive bile culture in cholelithiasis patient.

Bile culture status	No. of cases	Percentage
Positive	20	40%
Negative	30	60%

Table 5: Distribution of bile positive cases according to the type of gallstone.

Tyme of coll	No. of cases of cholelithiasis			
Type of gall stone	Bile culture	Bile culture	Total	
	positive	negative		
Cholesterol	2	13	15	
Pigment	0	4	4	
Mixed	15	10	25	
Combined	3	3	6	
Total	20	30	50	

Chi square test value-11.528, df-3, p<0.01

Table 6: Microrganism isolated from bile in cholelithiasis patient.

Bacteria isolated	No. of cases
E.coli	14
Klebseilla	4
Staphylococcus aureus	1
Salmonella typhi	1
Total	20

DISCUSSION

Cholecystitis and Cholelithiasis are prevalent in certain region of the world and quite rare at other places and has been reported in 54% of the adult above 21 years of age. The estimated prevalence of gallstone disease in India has been reported as 2% to 29%. In India this disease is seven times more common in north than in south India. Dietary differences in two regions are suspected to be responsible for the differences in prevalence rate. 8-10

The average age of these patients in India is a decade younger than those in western. The maximum no. of patient in this study was in the 3rd and 4th decade of life. Zuhair et all reported peak age of incidence as 41-50 years, Ranshoff and Gracie and one more study reported same results. 11-12

Studies have shown an increase in the prevalence of gallstone with age probably because of decrease in activity of cholesterol reductase and increase in activity of HMG CoA reductase. ^{13,14}

Male to female ratio in this study was 1:4, in all literature available so far females have been easiest victim of gallstone disease. In fact, female sex harmone and sedentary habits of most women in India expose them to factors that possibly promote the formation of gallstones. ^{11,15,16}

In this study gallstone was classified according to their morphological appearance and cases with mixed type of gallstone was found to be predominant 50%, followed by cholesterol stone 30%. Cases with combined type of gallstone were 12% and cases of pigment stone was 8%.

Other studies have also reported similar incidence of type of gallstone, however literature from Chennai, Jayanthi V et al were in contrast to our study with maximum cases of pigment type of gallstone. ^{13,17}

The variation in different types of gallstones varies significantly in different parts of India. Food habit may be one of the reasons. In this study, the positive bile culture was 40%, which is considerably higher than reported by Yaqin and sultan. Other studies show this positivity ranges from 16.4% to 46%. 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

kind of gallstone present and found more in mixed type of gallstone. 18-21

Bile in normal patient is found to be sterile, however in about 30-40% of the patient with cholelithiasis bacteria can be cultured from the bile.²⁰

In this study incidence of bile culture positivity was more in the cases of mixed type of gallstone (15/25) 30%, and the most common organism isolated was *E.coli* followed by *Klebseilla*. In other study it is found that mixed stone is frequently associated with cholecystitis. In Ohdan H et al study incidence of positive bile culture was 38% and 83% in mixed type o stone and *E.Coli* being the common organism isolated.²² It is also shown in other studies that Bacteria are regularly found in bile of patients with brown pigment gallstones 53%, 91%, 100% especially in cases of choledocholithiasis and hepatolithiasis. Bacteria are less often found in the bile of patients with pure cholesterol gallbladder gallstones 24% and black pigment gallstones 9% to 19%. ^{23,24}

E.coli was found to be the commonest organism in this study, and it also reported in previous studies. However *Klebsiella pneumonae* is reported by sabir. Attila cescend et al (52%), Willis RG. Stewart L et al (44%), Balla et al (46%) study all reported *E.coli* the commonest bacteria isolated. ^{20,25-27}

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.

Different factors have been implicated in the causation of gallstone among which infection of bile is also as important factor. The biliary infection can be caused by any type aerobic gram positive to gram negative to anaerobic organism. Aerobic organism causes 94% of biliary tract infection while anaerobic the rest. Bacteria are commonly found in inflamed gallbladder and in patient with cholelithiasis, whereas evidence suggests that normal bile are sterile. ²⁸⁻³¹

CONCLUSION

The frequency of bile infection in this study area was 40%, in which association with mixed type gallstone was found to be significant and the most common bacteria isolated was *E.coli* followed by *Klebseilla pneumonae*. So spillage of bile during cholecystectomy may lead to sepsis. Also culture of bile at Cholecystectomy is helpful because appropriate antibiotic can be administered in cases of culture being positive, hence avoid serious complication.

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Ethical approval: The study was approved by the

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