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Bacteriological study of nidus of gallstones

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ABSTRACT

Background: Gallstone disease is common worldwide and majority of patients undergo surgical management. Since the advent of laparoscopic cholecystectomy surgeons has become less interested in its aetiology. Infection is a major factor in the formation of gallstones. There are many infective complications reported of gallstones spilled during cholecystectomy. Presence of bacteria or its DNA by microscopy does not prove the infective potential of such stones. Thus culture of the nidus of the gallstone is the gold standard method to identify any potentially infective bacteria within and to predict the infective potential of gallstones.
Methods: Cross sectional study done in seventy patients admitted for elective cholecystectomy for a period of 24 months from August 2016 to July 2018 in Department of Surgery, Regional Institute of Medical Sciences, Imphal, India.
Results: Of the 70 stones cultured for bacteria, 52 (74.3%) were sterile and 18 (25.7%) were positive for bacteria, among which 16 stones showed mono-microbial growth while 2 showed poly-microbial growth. Most commonly isolated organism was *E. coli* and *Klebsiella* followed by *Pseudomonas, Enterococcus* and *Staph aureus*.
Conclusions: Moynihan's aphorism "gall stone is a tomb erected in the memory of the organism within it", suggest bacteria inside gallstone to be dead but this study proves that bacteria can be viable inside gallstone and its complete retrieval should be done in case of spillage which may help in avoiding complications.

Keywords: Gallstone bacteriology, Stone culture, Nidus of gallstone

INTRODUCTION

Gallstone disease is common worldwide, and its prevalence has geographical and ethnic variations.[1] In Asian countries, the prevalence of gallstone disease ranges from 3% to 10%. The prevalence of gallstone disease is 3.2% in Japan, 10.7% in China, 7.1% in Northern India, and 5.0% in Taiwan.[2-5] Gallstones are responsible for more than 95% of biliary tract disease.[6] Gallstone disease thus makes a considerable economical impact in healthcare scenario around the world even though the mortality associated with it is very less.[7-10]

Since the advent of laparoscopic cholecystectomy surgeons have become less interested in the aetiology and classification of gallstones, and an unjustified increase in surgical procedures has been observed as a laparoscopic cholecystectomy can be performed regardless of the aetiology or composition.[1] This practice has lead to further additional economical impact on the healthcare systems. Pathogenesis of gallstone disease is multifactorial. Among different factors causing gallstones formation, biliary infection can be found in a sizeable proportion of patients. Biliary infection can be due to gram negative, gram positive or anaerobic organisms.[1]

Bacterial infection is of great significance in the development of inflammation in gallstone disease. In normal subjects, bile is sterile as it has bactericidal activity. When there are changes in bile composition or cholestasis in the gallbladder, bacteria can rise into the gallbladder through the bile duct and promote lithogenesis and some bacteria may get entrapped within the core of gallstone during the process.[10] Such bacteria were long thought to be dead as said by the Lord Moynihan.

Treatment of gallstone doesn't depend much on its type, composition or the presence of bacteria within. But it was noticed that calculi spilled and gone missing in peritoneal cavity during cholecystectomy caused a variety of complications and peritoneal lavage and retrieval of spilled stones prevented the many complications associated with it.[11] This made many investigators think about the possibility of live bacteria inside the gallstone. Many studies have already proven the presence of bacteria inside the gallstones by a variety of techniques such as gallstone culture, scanning electron microscopy and molecular genetic techniques. Presence of bacteria in different types gallstones have been confirmed by all three methods mentioned above, in multiple studies done in this regard.[11-16]

But evidence of mere presence of bacteria or its DNA by SEM or molecular genetic techniques does not implicate the infective potential of the bacteria, which are thought to be responsible for many of the complications reported. In such scenario culture of the nidus of the gallstone remains the gold standard method to identify any potentially infective live bacteria within gallstone irrespective of its type or composition.

The current study is aimed at determining the trend of bacteriology by culturing live bacteria from the nidus of gallstone in patients undergoing cholecystectomy in an elective setting at a tertiary care centre in North East India and the results were compared with previous similar studies after correlating with age, sex and type of stone.

METHODS

Study design and duration

The present study was a cross sectional study conducted for a period of 24 months from August 2016 to July 2018 in the Department of Surgery, Regional Institute of Medical Sciences, Imphal, Manipur, India.

Study population and sample size

Seventy patients admitted to Department of Surgery, with diagnosis of gallstone disease for elective cholecystectomy were selected for the study.

Inclusion criteria

Patient of aged 18-70 years, all proven cases of gallstone disease who gets admitted for elective cholecystectomy.

Exclusion criteria

Acute cholecystitis, acute acalculus cholecystitis, empyema of gallbladder, mucocoele of gallbladder, gallstones with multiple common bile duct stones

Procedure

A thorough clinical examination was done and the findings were noted. The patients were subjected to an ultrasound examination of the abdomen and the presence of cholelithiasis was confirmed and associated acute or chronic cholecystitis was also noted. Patients who did not have cholelithiasis on USG were excluded from the study.

Detailed history of every patient with special emphasis on pain in right hypochondrium, nausea, vomiting, fever and jaundice was taken. Complete physical examination was done, and the findings of each patient were entered in to the standard proforma.

Ethical issues

The study was carried out with approval from the Research Ethics Board (REB), Regional Institute of Medical Sciences, Imphal.

Sample collection

Gall stones: After cholecystectomy gallbladder was cut open and the single largest stone was transferred to sterile culture bottle containing normal saline and was transferred to laboratory for stone culture.

Retrieved stones, were divided into two groups based on the colour and appearance of gallstones. Whitish to pale yellow and dirty yellow as cholesterol stones and black to blackish brown as pigment stones.

In laboratory whole stone was immersed in 70% ethanol for 10 min for surface sterilization, then it was air dried, bisected (taking sterile precaution) and the core was scooped out for culture. Culture was carried using recommended culture media.

Outcome measures: Culture positive samples of gallstone nidus.

Study variables

Age, sex, type of gallstone.

Statistical analysis

Data was compiled in IBM SPSS Statistics Ver. 22 and was checked for its completeness and correctness. Statistical analysis was done by chi square test and t test. P value >0.005 was taken as statistically significant.

RESULTS

Bacterial culture and analysis of seventy patients who underwent elective cholecystectomy is done and the results are as given below. Among the subjects sixty (85.7%) were females while males were ten (14.3%). Age group distributions of subjects are shown in Figure 1.

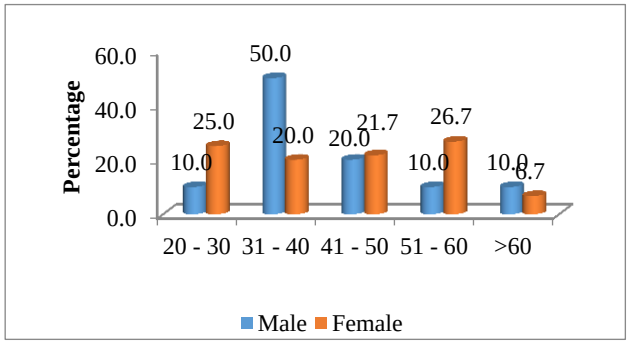

Figure1: Distribution of age and gender.

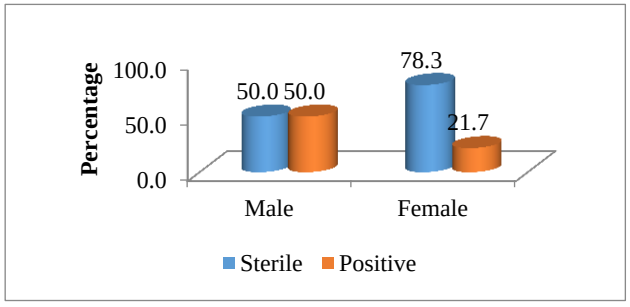

Figure 2: Comparison of sex based on cultural positivity.

Mean age of subjects in the study is 42.6 and the number of patients in each age group is not significantly different except the subjects above sixty years are five (7.1%).

Also ten out of seventy subjects were having either diabetes mellitus or hypertension and the rest of the patients were not having any associated comorbidities. Half of the subjects underwent laparoscopic cholecystectomy (35/70) while rest of the subjects underwent open cholecystectomy (35/70).

Among the seventy stones retrieved and classified, thirty (42.9%) stones were pigment stones and forty (57.1%) stones were cholesterol stones by the classification criteria used (Table 1).

Table 1: Percentage distribution of the sample according to type of gallstone.

Type of gallstone	Count	Percentage (%)
Pigment	30	42.9
Cholesterol	40	57.1

Table 2: Percentage distribution of the sample according to stone culture positivity.

Stone culture	Count	Percentage (%)	Odds (95% CI)
Sterile	52	74.3	15.5 - 35.9
Positive	18	25.7	

Table 3: Percentage distribution of the sample according to organisms identified.

Stone culture sensitivity	Count	Percentage (%)
Sterile	52	74.3
E. coli	6	8.6
Pseudomonas	4	5.7
Klebsiella	6	8.6
Enterococcus	3	4.3
Staph. aureus	1	1.4

Table 4: Comparison of type of gallstone based on cultural positivity.

Type of gallstone	Sterile		Positive		χ^2	P value
	Count	Percentage (%)	Count	Percentage (%)		
Pigment	23	76.7	7	23.3	0.16	0.693
Cholesterol	29	72.5	11	27.5		

Table 5: Comparison of age based on cultural positivity.

Age (in years)	Sterile		Positive		χ^2	P value
	Count	Percentage (%)	Count	Percentage (%)		
≤35	20	80.0	5	20.0	0.66	0.415
>35	32	71.1	13	28.9		

Table 6: Comparison of type of organism with type of gallstone.

Type of organism	Pigment		Cholesterol	
	Positive	Percentage (%)	Positive	Percentage (%)
E. coli	2	28.5	4	30.7
Pseudomonas	1	14.3	3	23.1
Klebsiella	2	28.5	4	30.7
Enterococcus	1	14.3	2	15.3
Staph. aureus	1	14.3	0	0
Total	7	100	13	100

Table 7: Comparison of type of gallstone with type of culture.

Type of stone	Culture positive stones	Mono-microbial	Poly-microbial
Pigment	7	7	0
Cholesterol	11	9	2

Out of the seventy stones which were cultured for bacteria fifty two (74.3%) stones were sterile and eighteen (25.7%) stones were positive for bacteria (Table 2), among which sixteen stones showed mono-microbial growth while two stones showed poly-microbial growth. Both those stones with poly-microbial growth were cholesterol stones as per classification (Table 7).

Most commonly isolated organism was *E. coli* and *Klebsiella* (8.6%) followed by *Pseudomonas* (5.7%), *Enterococcus* (4.3%) and *Staph aureus* (1.4%) (Table 3).

Out of thirty pigment stones, twenty three (76.7%) were sterile and seven (23.3%) were positive for culture. And among forty cholesterol stones, twenty nine (72.5%) were sterile and eleven (27.5%) were positive for culture (Table 4).

Also culture positivity among patients aged more than thirty five years was 28.9% (13/45) and those aged below or equal to thirty five years was 20% (5/25) (Table 5).

The culture positivity among males and females were also analysed and the results were 50% gallstone culture positivity in male patients while the only 21.7% culture positivity among females (Figure 2).

Out of the seven culture positive PS two were positive for *E. coli* and two were positive for *Klebsiella* (28.5% each). *Pseudomonas*, *Enterococcus* and *Staph aureus* were isolated from one stone each (14.3%) each. All PS were mono-microbial on culture.

Of the eleven culture positive CS, two stones were poly-microbial. One stone positive for both *Klebsiella* and *Enterococcus* and other one positive for both *Klebsiella* and *Pseudomonas*. So the among the total thirteen organisms isolated from eleven culture positive CS, *E. coli* and *Klebsiella* accounted for four (30.7%) each. *Pseudomonas* found in three (23.1%) and *Enterococcus* in two (15.3%) of the culture positive CS stones.

DISCUSSION

Possibility of finding live bacteria or its remnants inside the gallstone has been a topic of discussion since the cases of lost stones in peritoneal cavity causing complications started reporting. The current study evaluated the presence of live bacteria by culture of nidus of the gallstone irrespective of the type of stone and analysed the bacteriological profile of gallstone nidus. Out of seventy patients included in the study, males were ten in number while females were sixty in number.

The age group classification of both the sexes were done and the maximum patients were in the age group 30-41and 51-60 which is both 24.3%. In our study sixty out of seventy patients had no comorbidities associated with gallstone disease while six patients had hypertension and four patients had diabetes mellitus. Out of the seventy, thirty five of them underwent laparoscopic cholecystectomy while the other thirty five underwent open cholecystectomy for various reasons like patient's choice, associated comorbidities, previous history of abdominal surgery and conversion to open due to severe adhesions.

The gross morphological classification used in the study divides gallstones into two types; cholesterol and pigment stones. Only the external appearance of the gallstone was taken into account as a guide in dividing the stones into two types.

According to the study 57.1% were cholesterol stones and 42.9% were pigments stones. The variation in different types of gallstones varies significantly in different parts of India. Food habits may be one of the main reasons. Cholesterol gallstones are predominant in the northern, eastern and western parts of India, while pigment gallstones are common in the southern region.[20-22] Present study also yielded similar reports, with predominantly cholesterol stones in north India compared to pigment stones in southern India as reported by Amin et al.[22]

The current study analysed a total of seventy stones out of which 25.7% were positive for bacteria. It was also found that 27.5% of the total cholesterol stones and

23.3% of the total pigment stones showed positive culture. A study by Steward L et al showed twenty three percent of cholesterol and sixty eight percent of pigment stones.[13]

This difference could be attributed to the fact that the current study was done in an elective setting excluding all acute or emergency conditions and cholecystectomy done for other conditions like associated malignancy, CBD stone etc. It should be noted that the current study evaluated only the nidus of gallstone while Steward L et al assessed the outer pigment parts of certain stones which were classified as mixed or composite stones.[13]

Ramteke et al reported about 20% stone culture positivity with 4.7% culture positive cholesterol stone, 25.9% culture positive pigment stones and 100% positive mixed stone culture.[23] The morphological classification used here is different from the current study. The percent of culture positive stones is comparable to the present study. But the percentage positivity was different between various types of stones in both the studies possibly because of the different classification system used.

Hazrah et al in a study of gallstone has reported 81% culture positive stones, the highest percent in similar studies done.[11] It could be argued that they included patients with acute gallbladder conditions, CBD stones, gallbladder carcinoma in the study which is a notable difference from the present study. But still the possible reason for such a difference in the rate of culture positivity between the two studies could not be ascertained.

Leung et al reported about 80.6% culture positivity and only pigment stones were included in study.[17] Since pigment stone pathogenesis are proven in the past for their association with biliary tract infection this difference could be justified and the positive culture rates here are similar to the study by Hazrah et al.[11]

Steward et al shown that 23% of cholesterol and 68% of pigment stones contained bacteria and the criteria for identifying positivity in the study were SEM along with gallstone culture.[13]

Swidinski et al reported that bacterial growth was recorded in the culture in nine of hundred gallstones.[12] Polymerase chain reaction however detected bacterial DNA in eighty two of ninety one sterile gallstones. This again proves the higher positivity rates in molecular genetic methods compared to gallstone culture methods even though they don't prove the viability of the organism.

Leung et al in another study showed gallstone cultures were positive for sixty nine of the seventy (99%) stone samples with a total of one hundred and forty nine bacteria isolated.[18] Also thirty three different species of bacteria were identified among the bacteria isolated. All the patients were having acute cholangitis when included in this study which may be reason for such a high rate of gallstone positivity.

One drawback of the present study is the method of gallstone culture, which was done from the nidus (centre) of the stone only while it was shown by Steward et al some stones with outer pigment layer had bacteria in the pigmented periphery than in the nidus.[13] Also the classification of gallstone based on external appearance alone which was used in the current study was different from the classification used in other studies as mentioned above and the author feels that assessing the composition of gallstone using X-ray crystallography or infrared photometer and analysing the cut surface using SEM to find out the pattern of arrangement of cholesterol crystals and its layering with pigments for classification of gallstone by morphology is still the better classification. Even though studies done by using such classification, found it difficult to classify certain gallstones despite all these assessment, the author feels that the classification used in this study was a simplified one for gallstone classification. These points out a need in improvisation of the classification of gallstone as opined by Kim I et al.[19]

Among the organisms isolated in the current study both enteric and non-enteric organisms were present. Most common was *E. coli* and *Klebsiella* followed by *Pseudomonas*, *Enterococcus* and *Staph aureus* respectively. Studies by Steward et al showed most common as *Pseudomonas* followed by *E. coli*, *Klebsiella*, *Enterobacter* and *Enterococcus*.[13] Sushma et al showed 90% *E. coli* and rest *Klebsiella*.[23] Syed et al reported similar frequency of organisms as that of present study.[14] Hazrah et al reported *Klebsiella* as most common followed by *E. coli* and *Pseudomonas*.[16]

The culture positivity in males was about fifty percent while in females it was twenty one percent. Also the rate of gallstone nidus culture positivity in patients aged more than is twenty eight percent while its only twenty percent in patients younger than thirty years.

Thus it became clear that bacteria can remain viable inside the gallstones contrary to the Aphorism of Lord Moynihan.[24] The current study attempted to analyse the bacteriology of nidus of gallstone and it was found that one fourth of the patients were positive for bacteria with majority of the identified organisms being enteric gram negative bacteria. Actual incidence may be higher owing to the fact that many of the organisms that were reported from similar studies in the past require fastidious culture requirements which might have resulted in false culture negative reports which might have affected the outcome measured in the study. Thus it is understood that surgeons should be careful not to spill or drop gallstones intraperitoneally and the every attempt should be made to remove all the stones during cholecystectomy. Steward et al has shown cholesterol stone bacteria induce fewer complications than pigment stone bacteria.[13] This may

help the surgeon in predicting the various complications and its better management if they assess and identify the type of gallstone according to the classification systems. Also it should be borne in mind that the present study evaluated only the nidus of the gallstone while an infective complication on gallstone spillage during surgery can be caused even by bacteria in outer layers of gallstone also if present.

CONCLUSION

Bacteriological analysis of gallstone nidus was positive in one fourth (25.7%) of the patients undergoing elective cholecystectomy and the most common organisms identified were *E. coli* and *Klebsiella* followed by *Pseudomonas*, *Entercocci* and *Staph. aureus*. This study also shows that bacteria can remain viable inside gallstone and can be readily cultured from nidus of gallstone and may have the potential to cause infective complication contrary to what the Lord Moynihan has stated.

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