Original Article

Frequency of Bile Bacteria in Gallstone Disease

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Abstract:

Introduction: Gallstone disease is among the bread and butter surgery for most general surgeon but role of bacteria in its formation is still an enigma.

Methods: Bile was obtained during cholecystectomy from the patients admitted for uncomplicated cholecystectomy which was cultured in standard culture medium. Demographic profile and nature of stones obtained were also compared.

Results: This study involved microbiological analysis of bile obtained from 528 patients undergoing cholecystectomy without other complication for duration of one year in BP Koirala institute of health sciences, dharan. Study showed that incidence biliary infection was 4.8% with commonest organism being E coli followed by K pneumonia. Among various types of stones pigment stone were mainly found to be associated with infection than other types of stones.

Conclusion: The study shows that though biliary infection in patients with gallstone disease is not uncommon but its exact role in the pathogenesis of gallstone disease is not elusive.

Keywords: gallstone disease, biliary infection, pigment stones

Introduction

Gallstone disease is one of the common surgical condition requiring surgery in elective setup. The incidence varies widely across geographical regions with highest in the Scandinavian countries, Chile and among Native Americans and lower in sub-Saharan Africa and Asia.[1] The pathogenesis of gallstones is complex. Gallstone formation appears to be a complex trait influenced by genes and environment, and their interaction.[2] Genetic effects accounts for 25%, shared environmental effects for 13% and unique environmental effects for 62% of the liability.[3]

Among the important environmental factors in the pathogenesis of gallstones, is the involvement of bile bacteria. Various literatures report that bacterial infection is important mainly in the pathogenesis of pigment stones. They usually cause brown or black stones. Bile culture might be positive, yet no bacteria may be found in the centre of stones on scanning by electron microscope, even then it is helpful to prevent future stone formation. Certain studies have now established the role of certain bile bacteria like Helicobacter pylori in the formation of cholesterol gallstones.[4,5] But yet confirm and details of organism responsible for this common surgical enigma is still not lucid.

The objective of the study was to assess the rate of bile infection in patients with gallstone disease presenting for cholecystectomy.

Methods

This was a prospective observational study done in 528 patients undergoing cholecystectomy for gallstone disease which included all age group patients ranging from 7yrs to 87 yrs for a period of 1 year at BPKIHS, Dharan. All of these patients had preoperative diagnosis of cholelithiasis and were operated either laparoscopically or conventional

open technique. Patients with isolated choledocholithiasis, gallbladder mass or growth and those in whom emergency cholecystectomy done were not included in the study. Similarly those preoperatively on antibiotics for any reason were also not included.

The patient was admitted night before surgery in the concerned department and were examined and investigated by concerned surgical team and anesthetic team for fitness for surgery. After excluding those with above criteria rest were included in the study. Preoperatively all patients were given a dose of antibiotics (either ceftriaxone 1 gm or ofloxacin 200mg intravenously) at the time of induction. After exposure of gall bladder bile was aspirated aseptically and sent for culture in a disposable syringe. The bile received in microbiology department is the cultured in appropriate media for 48 hrs in an incubator. Result of positive culture was reviewed by resident and senior faculty member. Rest of cholecystectomy was carried out according to standard surgical procedure.

Post operative care was done as per surgical protocol and they were watched out for fever, abdominal distension, prolong paralytic ileus and any signs of sepsis. Patients were followed up in surgical OPD 1 week after discharge and as per required. All data obtained was entered in Microsoft Excel program and statistical analysis was done using SPSS 18 and EPI 6. Data collected were compared by student unpaired t test for means of normally distributed variables between the two groups, Standard deviation of means between the two groups and chi square test to compare proportion of two groups. One way ANOVA test was used to analyze data within same group and p value less than 0.05 was considered significant.

Results

During one year period total of 630 cholecystectomies were performed in BPKIHS which included 520 females and 110 males (figure 1) with commonest age group of 26-35 yrs (28.09%). Study showed that gallstone disease is common in reproductive age group females; p = 0.015 (p <0.05) (significant).(table 1)

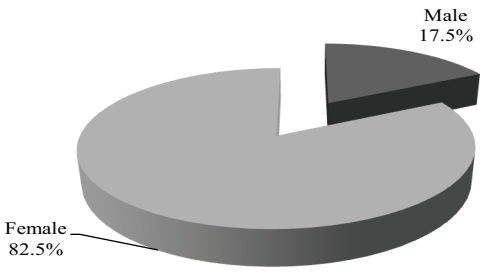

Figure 1 Gender distribution

Table 1: Age and Gender distribution

Age group (years)	Gender		
	Females	Males	Total
<15	5	2	7
16-25	77	4	81
26-35	148	29	177
36-45	137	23	160
46-55	82	23	105
56-65	59	21	80
>65	12	8	20
Total	520	110	630

The mean age of females presenting with gallstone disease in the study was 39.59 ± 10.4yrs while the mean age in males was comparatively more i.e., 45.43 ± 16.7yrs which is statistically significant p<0.005.

Table 2: Bile culture results

Organism	No of patients	Percentage
Escherichia coli	10	1.6
Klebsiella pneumoniae	6	1
Enterococcus spp	5	0.8
Coagulase negative Staphylococcus	3	0.5
Acinetobacter spp	2	0.3
Pseudomonas aeruginosa	2	0.3
Streptococcus spp	2	0.3

In total 630 cholecystectomies only 528 were sent for bile culture. Remaining 102 cases were excluded as in some cases bile spilled during cholecystectomy, few were lost during transport, few couldn't be done due to financial constriaints and in some bile couldn't be retrieved aseptically. Out of 528 bile cultures sent for microbiological analysis only 25 (4.8%) were positive with Escherichia coli (1.6%) as the commonest organism followed by Klebsiella pneumoniae, Enterococcus. (Table 2) Gram negative rods were predominant organisms of stone formation in the gall bladder followed by gram positive cocci.

On correlating different type of stones with infection of bile, pigment stones were found to be more infected than other stones. (Table 3) Pigment stones are associated with infection which was statistically significant to other stones. Our study showed commonest organism in cholesterol stones as E coli (7/19) while in pigment stones commonest organism isolated was K pneumoniae (3/7) and in mixed stone multiple organisms were isolated (E coli, K pneumoniae and Enterococcus spp).

Table 3: Bile culture and type of stone

Bile culture	Type of stone			Total
	Cholesterol	Mixed	Pigment	
Sterile	448 (80.87%)	37 (69.81%)	18 (78.26%)	503
Infected	18 (3.25%)	2 (3.77%)	5 (21.73%)	25

p = 0.000, significant

This shows that 21.73% of all pigment stones were infected with organisms in comparison to cholesterol and mixed stones, 3.25% and 3.77% respectively.

On review of the history of the patients, 17 patients having recurrent biliary colic had positive bile culture which is not significant statistically.(p= 0.907) In the patients with positive bile culture (25/528) only 1 had fever (out of 25), 1 had icterus (out of 25) and 5 had positive signs of hypertriglyceridaemia (out of 25). Thus it is unlikely to preoperatively judge a patient to have infected bile based on history and physical finding.

Table 4: Recurrent biliary colic and bile culture

Recurrent attacks		No	Yes	No of patients (n)	P value
Bile culture	Sterile	130	373	503	0.907
	Infected	8	17	25	NS

Among the patients included in the study patients undergoing open cholecystectomy had more number of positive bile cultures than those undergoing laparoscopic cholecystectomy and converted cases. This doesnot signify that patient with infected bile culture tend to have difficult morphology or anatomy.

Table 5: Bile culture and type of operation

	Laparoscopic	Conversion	Open	Total
Positive bile culture	7	1	17	25

Among 25 patients with positive bile culture 1 patient had prolong paralytic ileus (>24hrs) while 1 patient developed mild fever which subsided after couple of days prolonging her hospital stay. In rest it was uneventful hence not signifying any major event.

Discussion

Gallstones are very common in females as they are genetically susceptible; have decreased physical activity level, involvement of female hormones and dietary factors. Our study too gallstone disease was common in females than in males (82.5% Vs 17.5%). In a study by Pancorbo et al prevalence of gallstones was higher in females (11.5%, 95% CI 8.2 – 14.7) than in males (7.8%, 95% CI 4.6 – 11.1).[6]

Our study showed that younger females (mean ± SD) (39.59 ± 10.4) years had gallstone disease more often as compared to males (mean ± SD) (45.43 ± 16.7) years which was statistically significant (p=0.015). The gallstone disease was common in child bearing age i.e., between 26 to 45 years in both gender which is 50% of total study population. This is probably due to involvement of female hormones. Gallstone disease was seen in males in older age than females where 50% of study population is between 26 – 45 yrs while in males 50% of study population is between 26 – 55yrs. With increasing age males also tend to have decreased level of physical activity hence predisposing to gallstone formation. A study by Kalina Z et al also showed that prevalence of gallstone is higher in females and in both sex its incidence increases with age.[7] Similarly Channa et al in 2004 reported that the peak age group for the occurrence of gallstones in males was 45-59 years and in females 30-44 years which is close to our study.[8]

Positive bile culture was obtained in 25/528 (4.74%) cases with Escherchia coli (1.6%) as the commonest organism followed by Klebsiella pneumoniae (1%) and Enterococcus spp (0.8%). Only 528 samples of bile was sent for analysis despite the study group of 630 as in some cases bile couldn't be obtained, in few bile was lost during transport and the rest could not send bile for culture due to financial constraints. The size of stones 10 to 20 mm were more commonly infected than other stones (10/25) by Escherchia coli. In stones larger than 20 mm, Klebsiella pneumoniae was the commonest organism present (9/25). Pigment stones were found to be commonly infected 5/23 i.e. 21.73% than other types of stones viz cholesterol (3.25%) and mixed (3.77%). In patients with positive bile culture one had fever, one had icterus and 5 had signs of hypertriglyceridaemia. Most of the patients undergoing open cholecystectomy had positive bile culture (17/25). Lygia Stewart et al found out that bacteria was present in 75% of pigment stone, 76% of mixed stone and 20% of cholesterol stones.[9] In a study by Kaufmann et al bile cultures were positive in 13% of patients with cholesterol stones, in 14% of those with pigment stones and in all of the patients with brown

pigment stones ($p < 0.005$).[10] In one of the studies in Saudi Arabia, the most common organism isolated were E coli (28.1%), Enterococcus faecalis (15.6%) and Pseudomonas aeruginosa (9.4%).[11] An Indian study showed E. coli (45.07%) and Klebsiella (25.35%) as predominant among the aerobes and Bacteroides fragilis (58.82%) among the anaerobes.[12] In a study by Irfan Sattar et al 36% had positive bile culture with E. coli as commonest organism followed by Klebsiella, Pseudomonas and Staphylococcus aureus.[13]

One hundred and fifty-two consecutive cases of cholelithiasis were studied by Malatani Tarek S and et al to determine the constituents by culturing the bile specimen and correlating with the wound infection. It has shown that mixed gallstones were the commonest type (58.5%), pigment stones (27%) and cholesterol stones (14.5%). Positive bile cultures were found in 41 patients (27%) and Escherichia coli was the commonest organism isolated. The rate of wound infection was 14.5% and the most common organism cultured from the wound was staphylococcus epidermidis.[14]

Conclusion

Among biliary infection E coli was found to be the commonest organism followed by K pneumonia and probably is the most common source of pigment stones. Gallstones with infected bile are not uncommon entity and aseptic precaution should be taking while removing it. This study is however inconclusive to determine it exact role of bile bacteria in the genesis of gallstone which might require more sophisticated techniques and modern diagnostic methods to identify them.

Conflict of interest: None declared.

References

1. American Gastroenterological Association website. Available at: www.acg.gi.org
2. Shaffer EA. Gallstone disease: Epidemiology of gallbladder stone disease. Best Practice and Research in Clinical Gastroenterology 1920;(6):981-996
3. Wittenburg H, Lammert F. Genetic predisposition to gallbladder stones. Semin Liver Dis. 2007;27:109-121
4. Cetta F et al. The Role of Bacteria in Pigment Gall bladder Stones; Ann Surg 1991 Apr; 213 (4): 315-26
5. Krik J. Maurer, Arlin B. Rogers, Zhongming Ge, Ashley J. Wiese, Martin C Carey, James G. Fox. H. Pylori and Cholesterol gallstones formation in C57L/J mice: a prospective study; 2006 American Physiological Society; Am J Physio Gastrointest Liver Physio 2006; 290: G175- 82
6. Pancorbo Carmen et al; Prevalence and associated factorsw for gallstone disease: Results of a population survey in Spain; J Clin Epidemio 1997- 50 (12) 1347-55.
7. Kalina Z, Szwed Z, Wanat-Wiśniewska M, Waleczek J, Hausner L, Tokarz D; Epidemiology of cholelithiasis among professionally active persons in the Katowice province; Wiad Lek. 1992 Apr;45(7-8):251-4
8. Naseem A. Channa, Fateh D. Khand et al: Surgical incidence of cholelithiasis in Hyderabad and adjoining area. Pak J Med Sci. 2004; 20 (1), 13-7
9. Lygria stewart, J McLeod Grifiss, Gary A Jarvis, Lawrence W Way. Biliary bacterial factors determine the path of gallstone formation. The Am J of Surg 2006 192; 598-603
10. Kauffmann Howard, Thomas Magnuson, Keith Lillemoe, Peter Frasca and Henry Pitt. The Role of Bacteria in Galbladder and Common duct stone formation. Annals of Surgery may 1989, 209 (5) 584-591
11. Al Harbi M, Osoba AO, Mowallid A, Al-Ahmadi K.Tract microflora in Saudi patients with cholelithiasis. Trop Med Int Health 2001; 6: 570- 4
12. Ballal M. Bacteriological spectrum of cholecystitis and its antibiogram. Indian J Med Microbiol 2001; 19: 212 -4
13. Irfan Sattar, Adnan Aziz, Shahid Rasul, Zahid Mehmood and Asadullah Khan; Frequency of infection in cholelithiasis; JCPSP 2007, Vol. 17 (1): 48-50
14. Malatani Tarek S, Bobo Raymond A, Al-Kassab Abdul Salam, Al-Saigh Abdulaziz S, Ajao Oluwole G, Jastaniah Suleiman, Bhattachan Chitra L. Gallbladder stones analyzes, bile and wound cultures in cholelithi asis.1996;vol2(3);146—149