Immunopathologia Persa
http www.immunopathol.com
DOI:10.34172/ipp.2022.27261

Evaluation of gallbladder microorganisms in patients undergoing cholecystectomy and its relationship with clinical and laboratory parameters

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Received 11 June 2021
Accepted 3 Sep. 2021
Published online 8 Jan. 2022

Keywords: Acute cholecystitis, Chronic cholecystitis, Gallstones, Microbiome

Abstract

Introduction: Acute cholecystitis is one of the most common gallbladder diseases. Surgical operation is still the main treatment of biliary diseases, but there may be required a period of stabilization in the hospital before cholecystectomy. Infected bile in different studies has been reported from 50% to 75%. Therefore, identification of microbial mass followed by antibiotic treatment for this disease is important.
Objectives: In this study, bacterial spectrum in patients undergoing cholecystectomy was evaluated based on clinical symptoms and laboratory parameters.
Patients and Methods: In this prospective cross-sectional study, all patients requiring cholecystectomy were enrolled in the study. In the study, the data on age, gender, clinical signs of all patients and laboratory results and biliary liquid experiments were collected.
Results: Fourteen cases including 13 cases of *E. coli* and a *Staphylococcus* strain had positive cultures. No significant correlation was detected between gender, fever, vomiting, tenderness right upper quadrant (RUQ), anorexia, jaundice, age group and microbial growth ($P>0.05$). Moreover, no significant correlation between laboratory parameters such as white blood cell (WBC), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, amylase and microbial growth was seen ($P>0.05$).
Conclusion: Bile culture was positive in 14% of patients undergoing cholecystectomy and *E. coli* was the predominant microorganism. Frequency of positive culture is low and has no relationship with demographic, clinical and laboratory factors and could not have a significant effect on the occurrence of postoperative complications; therefore, it seems that the administration of antibiotics before surgery is not necessary.

Citation: Hemmati HR, Parian P, Molaei A, Izadi S, Mirmohammadkhani M, Soltani S. Evaluation of gallbladder microorganisms in patients undergoing cholecystectomy and its relationship with clinical and laboratory parameters. Immunopathol Persa. 2022;x(x):e27261. DOI:10.34172/ipp.2022.27261.

Introduction

Cholecystectomy is one of the most common elective surgeries known as the first line therapy for symptomatic gallbladder diseases (1,2). In 75 to 90% of patients, this operation can relieve and eliminate complete or almost complete preoperative symptoms (3).

Until 1986, cholecystectomy was performed only by open surgery (4), which resulted in some postoperative complications due to damage to the abdominal wall. In 1987, Philip Moore conducted the first operation with a laparoscope, which became so popular due to the popularity of this method; thereby, except in a few cases, it does not need to the abdomen incision. It has been effective in reducing postoperative complications (5,6).

The most common *indications for* cholecystectomy include acute and chronic cholecystitis and gallstones (cholelithiasis), which can be asymptomatic or symptomatic.

Key point

Infected bile have been reported from 50% to 75 %. Identification of microbial mass followed by antibiotic treatment for this disease is important in cholecystitis.

The most common symptoms of biliary tract diseases include constant or intermittent pain or tenderness in the right upper quadrant (RUQ)or epigastric region, nausea, vomiting and leukocytosis (3,7).

Some of the post-cholecystectomy complications may occur including, abscess formation (often below the diaphragm), internal or external bleeding, biliary-intestinal fistulas and bile leakage (3.7).

Biliary tract infection has been reported from 50% to 75% in various studies (8). The most common organisms isolated from bile cultures include *Escherichia coli*, *Streptococcus, Enterobacter, Salmonella* and

Clostridium (8). In stone-less cholecystitis, a microbial agent can also be present (3,8), which are unusual bacterial infections in the gallbladder, such as *Vibrio cholerae*, *Leptospira*, and *Salmonella* (3).

Although surgical intervention is the first line therapy in many biliary diseases and their complications; however, an inpatient stabilization period may be required prior to cholecystectomy (3). Approximately, in 75% of patients who were treated medically, acute symptoms resolve within 2-7 days after admission (3). In addition, postoperative complications subside in patients who received antibiotics (9).

A few studies have been conducted regarding gallbladder microbiological spectrum in patients undergoing cholecystectomy. It is very important to identify the microorganisms and prescribe an appropriate antibiotic. It should be noted that bile leakage and peritonitis are rare after open cholecystectomy, but increase in laparoscopic cholecystectomy (10). Due to the rapid popularity of laparoscopic methods and the prevention of these complications, it is important to identify these microorganisms.

Objectives

In this study, bacterial spectrum in patients undergoing cholecystectomy was evaluated based on clinical symptoms and laboratory parameters.

Patients and Methods

Study design

In the descriptive and prospective cross-sectional study, all patients referring to Amir Al-Mo'menin hospital in Semnan from September 1, 2013 to June 31, 2014 for cholecystectomy (open surgery or laparoscopic surgery for any reason) were enrolled.

Convenience sampling.

During the mentioned period, 124 patients referred for cholecystectomy. Patients, who had taken antibiotics before the operation and the cases in which the pathology sample for desired tests was not delivered to the laboratory at the appropriate time, excluded from the study and eventually 100 samples entered to final stage of the study.

Patients' characteristics including age, gender and clinical signs were recorded in the checklist. Blood samples were taken before the operation for laboratory tests. Gallbladder fluid samples were taken at operating room during surgery and then the samples were sent to the laboratory. Information about microorganisms such as *E. coli, Streptococcus, Enterobacter, Salmonella* and *Clostridium* or other microbial agents was obtained based on culture results and recorded in checklist.

For microbial culture, bile samples were obtained under sterile conditions during surgery. Then they were poured off in laboratory sample tube and sent to the laboratory immediately. The samples were incubated at 37°C. Microbiological findings were obtained for *E. coli, Enterobacter* and *Clostridium* based on culture on eosin methylene blue agar (EMB) plate, in the case of *Streptococcus* from blood agar plate and in the case of Salmonella from *Salmonella Shigella* (*SS*) *agar* plate by standard bacteriological methods.

Data analysis

The Wald method employed to express the prevalence of infection with microorganisms (95% confidence interval) and chi-square, Pearson's and Fisher's exact test was used for evaluating the relationship between clinical and laboratory parameters. Descriptive findings were reported as tables.

Results

Out of 100 patients enrolled in the study, 76 of whom were female. The mean age (±SD) was 45.5 ± 13.2 years (22-76 years). Table 1 shows the distribution abundance of bacteria in various age groups.

Fever, nausea & vomiting, RUQ tenderness, anorexia, and jaundice were seen in 17 (17%), 40 (40%), 91 (91%), 46 (46%), and 3 (3%) of patients, respectively. According to the results of microbial culture, we had only 14 cases of microorganisms' growth, of which 13 cases were *E. coli* and one case was *Staphylococcus*. There was no significant relationship between patients' age and growth of microorganisms ($P=0.936$).

There was no significant relationship between fever ($P=0.702$), nausea and vomiting ($P=0.724$), tenderness ($P=0.609$), anorexia ($P=0.799$) and jaundice ($P=0.367$) and the growth of microorganisms (Table 2).

The mean, standard deviation, minimum and maximum of White blood cell (WBC), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and amylase are listed in Table 3. There was no correlation between different laboratory parameters and microorganism growth (Table 4).

Discussion

Cholelithiasis is one of the most common gastrointestinal problems with a prevalence of 11% to 36%. The most common therapy is cholecystectomy (11).

The pathophysiological role of bacteria in the formation of gallstones has long been mentioned (12).

The rate of positive microbial culture of bile varies in

Table 1. The distribution abundance of bacteria in various age group

Age group (y)	Growth of microorganisms				Total	
	Positive		Negative			
	N	%	N	%	N	%
45 and younger	7	14.3	42	85.7	49	100
46 and older	7	13.7	44	86.3	51	100
Total	14	14	86	86	100	100

Table 2. The distribution abundance of bacteria based on clinical signs

Clinical Signs and Symptoms		Results of Culture				Total		*P* value
		Positive		Negative				
		No.	%	No.	%	No.	%	
Icterus	Yes	13	13.4	84	86.6	97	100	0.367
	No	1	33.3	2	66.7	3	100	
Fever	Yes	11	13.3	72	86.7	83	100	0.702
	No	3	17.6	14	82.4	17	100	
Anorexia	Yes	8	14.8	46	85.2	54	100	0.799
	No	6	13	40	87	46	100	
Tenderness in RUQ	Yes	2	22.2	7	77.8	9	100	0.609
	No	12	13.2	79	86.8	91	100	
Nausea and vomiting	Yes	9	15	51	85	60	100	0.724
	No	5	12.5	35	87.5	40	100	

Table 3. The distribution abundance of quantitative laboratory parameters

	Laboratory parameters					
	Amylase (U/L)	ALP (U/L)	WBC ($\times 10^3/mm^3$)	Bilirubin T (mg/dL)	AST (U/L)	ALT (U/L)
Mean	297.1	216.47	8.339	0.8695	33.85	38.699
Median	299.5	205	8.05	0.75	28	28
SD	146.82	87.34	2.583	0.5572	39.64	55.88
Minimum	53	68	3	0.3	8	10
Maximum	984	817	15.6	3.3	361	441

Table 4. Distribution abundance of bacteria based on levels of laboratory parameters

Laboratory parameters	Culture results	No.	Mean ± SD	*P* value
WBC ($\times 10^3/mm^3$)	Negative	86	8.37 ± 2.67	0.855
	Positive	14	8.16 ± 2.03	
Bilirubin T (mg/dL)	Negative	86	0.868 ± 0.54	0.866
	Positive	14	0.8786 ± 0.62	
AST (U/L)	Negative	86	35.25 ± 42.53	0.424
	Positive	14	25.21 ± 6.42	
ALT (U/L)	Negative	86	40.50 ± 59.84	0.382
	Positive	14	27.57 ± 14.36	
ALP (U/L)	Negative	86	217.06 ± 90.83	0.948
	Positive	14	212.78 ± 64.23	
Amylase (U/L)	Negative	86	296.01 ± 140.49	0.785
	Positive	14	303.78 ± 187.20	

different studies. This variability may be due to differences in the proportion of acute cholecystitis in different studies (13-15). The incidence of positive bile culture is higher in patients with acute cholecystitis, obstructive jaundice, diabetes mellitus and patients with immunodeficiency (16).

The results of this study showed that one in seven patients undergoing cholecystectomy had a positive biliary culture and this positive culture was not associated with any of the clinical and laboratory parameters in these patients.

In our study, in line with other studies, *E. coli* was the most common organism followed by enterococci, *Klebsiella* and *Staphylococcus aureus*. However, in our study, the most common organism after *E. coli* was *S. aureus*. It is noteworthy that the low-number of positive cultures in this study may be due to laboratory, racial or geographical variations or that polymerase chain reaction (PCR) methods should have been employed for more accurate results.

In the study by Ohdan et al, 38% of cases were positive for microorganism culture and the predominant organism was Escherichia coli, which was seen in 22% of cases, followed by *Klebsiella* 18%, enterococci 15% and anaerobes less than 4%. In our study, *E. coli* was the most common organism (17). However, they showed the cultivation positive rate was 14%, which is about one third of the rate mentioned in the study by Ohdan et al (17). The reason could be racial and geographical differences (17).

In the study by Abeysuriya et al, of 70 cholecystitis patients, 54% were culture positive (18), which is almost

four times more than our study. The predominant bacterium in their study, as in our study, was *E. coli*, followed by *Enterococcus, Klebsiella* and *Staphylococcus*. In our study, *Staphylococcus* was the second most common microorganism which was seen other than *E. coli*.

In the study by Mahafazah et al, of 1248 cholecystitis patients for whom bile culture was conducted, 250 patients (20%) were positive culture. The study showed, 53.6% were gram-negative microorganisms, 29.2% were gram-positive and 17.2% were a combination of gram-positive and negative microorganisms. In our study, the microorganism growth rate was less than the study by Mahafazah et al and also more than 90% of the microorganisms were gram-negative (19).

In the study by Lemos et al in Brazil, bacterial DNA was examined in 84 patients with acute and chronic cholecystitis. Based on PCR results, bacteria were found in 42 patients (50%) but in only 16 patients (19%) the culture was positive. One of the main reasons for the different results in various studies is the diagnostic method. According to methods such as PCR, more reliable results can be achieved compared to other methods such as culture in which the probability of microbial contamination and false positive and even negative results is higher than the PCR method (20).

In the study by Kim et al, the results of the bile culture and its relationship with laboratory data and postoperative infections showed that 40.7% of cultures were positive, along with high levels of alkaline phosphatase and total bilirubin values (21). In our study, the positive culture results were about one-third and there was not any association with alkaline phosphatase values and total bilirubin levels.

In the study by Veselov et al, the gallbladder microbiome and its antibiogram was assessed in 100 patients who underwent surgery for chronic cholecystitis due to gallstones and the results showed that 82% contained microorganisms. Gram-positive organisms, especially staphylococci, were the predominant microorganisms (22). The results of this study were different from the findings of our study and other studies mentioned earlier, which could be due to climatic and racial factors.

In the study by Mamatha Ballal et al, the bile culture results were positive in 187 cases (60.9%) and the most common microorganisms were Escherichia coli (44.4%) followed by Klebsiella pneumonia (27.3%). In anaerobic culture, *Bacteroid fragilis* was seen in five cases which indicated to higher growth of gram-negative bacteria in the samples (8). The results of this study were similar to our study, despite the small number of positive results for cultures, the most commonly observed microorganism was *E. coli*.

Conclusion

Bile culture was positive in 14% of patients undergoing cholecystectomy and *E. coli* was the predominant microorganism. Positive culture has no relationship with demographic, clinical and laboratory factors and could have a significant effect on the occurrence of postoperative complications. Due to the low-number of positive cultures, it can be recommended that the administration of antibiotics before surgery is not necessary.

Limitations of the study

The diagnostic method in our study was microbial culture employing precise methods such as PCR was not possible. Furthermore multi-center studies with larger sample size and utilizing precise methods, such as PCR are suggested to achieve more documented results in this area.

Authors' contribution

HRH, SS and AM were the principal investigators of the study. HRH, PP, SS, MM and SI were included in preparing the concept and design. HRH and SS revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Semnan University of Medical Sciences approved this study (Code: IR.SEMUMS.REC.1392.29). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from M.D thesis of Parisadat Parian at this university (Thesis #607).

Funding/Support

The Research Deputy of Semnan University of Medical Sciences supported this study (Grant #607).

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