

Original article

Bacteriologic Analysis of Bile in Cholecystectomy Patients

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ÖZET

Kolesistektomi yapılan hastalarda safra kültürünün mikrobiyolojik analizi

Amaç: Bu çalışmanın amacı, kolesistektomi yapılan hastalarda safra kültürünün mikrobiyolojik analizini yapmak ve safra kültüründe bakteri üremesini etkileyen faktörleri değerlendirmektir.

Materyal ve Metot: Prospektif ve çok merkezli olarak planlanan çalışmamızda, kolesistektomi uygulanan 114 hastanın safrası ameliyat esnasında steril şartlarda alındı, kültür ve antibiogram testleri uygulandı. Kültür sonucunda üreyen bakterilerin cinsi belirlendi. Kültür sonucu pozitif olan hastalar, yaş, cins, teşhis, yandaş hastalıklar ve postoperatif komplikasyonlara göre değerlendirildi.

Bulgular: Çeşitli sebeplerle kolesistektomi uygulanan 114 hasta çalışmaya alındı. En sık kolesistektomi sebebi kronik taşlı kolesistit (kolelitiasis)'tir. Hastaların 15'inde (%13,1) safra kültüründe bakteriyel üreme tespit edildi. En sık üreyen bakteri Enterekok türleri (4 olgu, %26,6), Escherishia coli (3 olgu, %20) ve Enterobakter (3 olgu, %20)'dir. En fazla üreme, koledok taşı ile birlikte olan akut kolesistitli hastalarda (3 olgu, %100) görüldü. Yaşı 60'dan ileri olanlarda (10 olgu, %27) ve yandaş hastalığı olan hastalarda üreme daha fazla (9 olgu, %23,6) görüldü. Tüm hastaların sadece birinde postoperatif cerrahi alan enfeksiyonu görüldü; safra kültürü pozitif olan hastalarda enfeksiyöz komplikasyon görülmedi.

Sonuç: Çalışmamızın sonucuna göre, safrada bakteri üremesini artıran bazı faktörler belirledik: 60'dan ileri yaş, yandaş hastalıklar ve safra stazı oluşturan durumlar. Üreyen bakteriler intestinal kaynaklıdır. Kolesistektomi yaralarında cerrahi alan enfeksiyon riski düşüktür.

Anahtar Kelimeler: Kolesistektomi; safra; kültür

ABSTRACT

Objective: The aim of this study is to do a microbiologic analysis of bile in cholecystectomy patients and to assess the factors that contribute to bacterial growth in bile culture.

Methods: This study that was designed to be prospective and include multiple centers. The bile of 114 patients that had a cholecystectomy was taken in a sterile environment during surgery and a culture with antibiogram tests was performed. Patients with positive culture results were reviewed according to the age, sex, diagnosis, additional diseases and postoperative complications.

Results: 114 patients underwent a cholecystectomy for various reasons were included in the study. Bacterial growth was detected in the bile culture of 15 patients (13.1%). The most commonly isolated bacteria were Enterococcus spp (4 patients, %26.6%), Escherishia coli (3 patients, 20%) and Enterobacter spp (3 patients, 20%). The bile culture positivity rate was highest in patients with acute cholecystitis combined with choledocolitiasis (3 patients, 100%).

The bile culture bacterial growth was highest in patients over 60 years of age (10 patients, %27) and in those with concomitant illness (9 patients, 23.6%).

Postoperative surgical site infection was detected in only one patient; there were no surgical site infections in patients with a positive bile culture.

Conclusion: We identified some factors that can increase the growth of bacteria in bile: age over 60, concomitant illness and some conditions causing stasis of bile.

Key Words: Cholecystectomy; bile; culture

INTRODUCTION

Cholecystectomy is currently a frequently performed operation. The most common reason for a cholecystectomy is gallbladder stones.

Gallstones usually led to chronic dyspeptic symptoms and sometimes can manifest as acute cholecystitis. Healthy individuals with no bacteria in the gallbladder sac can still have gallstones. In particular, if the gallbladder stones are pigment stones, bacteria is more likely to be present^{1,2}. In cases of acute cholecystitis, microorganisms in the bile multiply and lead to bacterial infection.

According to expected rates of infection, a cholecystectomy wound is classified as clean/contaminated (2nd class) and the rate of surgery area infection is between 2-9%³. Prophylaxis before

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surgery is given to prevent these infections. It is important to know the microbiological flora of the gallbladder before prophylactic antibiotics are given. Prophylaxis would be appropriate according to bacteria isolated from the bile and could prevent postoperative infection complications⁴.

The relationship between the presence of microorganisms in the gallbladder and postoperative wound infections has been shown in the previous studies^{5,6}. In these studies, surgical site infections were more frequent in patients with positive bile cultures. In the presence of predisposing factors such as acute cholecystitis, cholangitis, obstructive jaundice, diabetes and an age of over 60, bacterial growth was seen more frequently in bile cultures⁷.

The purpose of this study is to analyze bile cultures of 114 cholecystectomy patients with gallbladder disease and discuss the characteristics of patients with positive cultures.

MATERIALS AND METHODS

Our study was designed to take place between July 30, 2009 and November 30, 2010 as a prospective multi-center study.

The study included patients who had a cholecystectomy due to various gallbladder pathologies. Patients had surgery in the surgical clinics of Private Sema Hospital, Private Central Hospital, Pendik State Hospital and Darica Farabi State Hospital. Gallbladder illness was diagnosed by ultrasound or computerized tomography. Age and sex were not used to include or exclude patients from the study. All of the patients were given a single dose of Cefazolin 1 gram before the surgery as prophylaxis followed by two more doses after the procedure. Patients who needed antibiotics for a longer duration and those using antibiotics due to other illnesses were not included in the study. Three patients required open cholecystectomy surgery while the other 111 patients had laparoscopic cholecystectomy procedures. Additional diseases and postoperative complications of each patient were recorded.

Bile Collection and Culture Methods

During surgery, 2-3 ml of sterile bile was aspirated with a syringe from the gallbladder after the cholecystectomy and transported to the laboratory for culture and antibiotic resistance testing. Bile was inoculated into chocolate agar, blood agar and EMB mediums for aerobic culture testing. Growth was tracked for 48 hours. Once growth occurred in the medium, an antibiogram test was performed using the disk diffusion method. The types of bacteria were determined using an API 20 E (Biomerieux).

A chi-square test was used for statistical analysis with significance defined at $p < 0.05$.

RESULTS

A total of 114 patients were included in this study. Ages ranged from 17 to 86 years with a mean age of 48.6 ± 16.8 . 81 patients (71.1%) were women and 33 (28.9%) were men. Eleven of 15 of those patients with a positive bile culture were women (73.3%) and 4 were men (26.7%).

The ages of patients with a negative bile culture ranged from 17 to 83 years and the average age was 46.4 years. The ages of the 15 patients with a positive bile culture ranged from 30 to 86 years with an average age of 63.1.

One hundred eleven patients underwent a laparoscopic cholecystectomy and three patients underwent open cholecystectomy. In one of the three open cholecystectomy patients with bile duct stones and acute cholecystitis a cholecystectomy with T-tube drainage was performed. The other two were given a laparoscopic cholecystectomy followed by ERCP. The most frequent reason for a cholecystectomy was chronic cholecystitis and acute cholecystitis. Cholecystectomy reasons are shown in Table 1.

Table 1. The Number and Diagnoses of Cholecystectomy Patients

Cholecystectomy reasons	Number and rate (n=114)	Bile culture growth (n=15)
Chronic calculous cholecystitis (cholelithiasis)	94 (82.3%)	10 (66.7%)
Acute cholecystitis	16 (14.1%)	2 (20%)
Acute cholecystitis+ choledocholithiasis	3 (%2.6%)	3 (13.3%)
Polip of gallbladder	1 (0.8%)	0

Fifteen patients (13.1%) had bacterial growth in their bile cultures. The most commonly isolated bacteria were Enterobacter species, E. coli and Enterococcus spp. The types of isolated bacteria and rates are shown in Table 2. Anaerobic bacterial cultures were not performed in this study.

Table 2. The number and Rate of Bacteria Isolated in the Bile Culture

Growth bacteria in bile cultures	Number and rate
Enterobacter spp	4 (26.6%)
Escherichia coli	3 (20%)
Enterococ spp	3 (20%)
Pseudomonas spp	2 (13.3%)
Serratia fontiloca	1 (6.7%)
Stafilococ spp	1 (6.7%)
Streptococcus viridans	1 (6.7%)
Total	15 (100%)

Table 3. The Outcomes of Bacterial Growth in Patients with Co-morbid Diseases

Co-morbid Diseases	Patients with a positive bile culture	Patients with a negative bile culture	Total
Hypertension (HT)	7	2	9
HT + DM	1	2	3
HT+ (other diseases)	3	0	3
Diabetes (DM)	3	1	4
DM +(other diseases)	3	1	4
Cardiac diseases	3	0	3
Goiter (operated)	2	1	3
Gastric cancer (operated)	0	1	1
Other diseases	8	0	8
Total			38

In this study, ten of 38 patients over the age of 60 (27%) and five of the 77 patients under the age of 60 (6.4%) had bacterial growth in their bile cultures. When we evaluated the growth of bacteria in bile culture according to age, we found growth in the bile culture to be statistically significant in patients over 60 years of age ($p=0.006$).

In our study, the bacterial growth was found in only five out of 19 patients (26.3%) with acute cholecystitis (16 acute cholecystitis, 3 acute cholecystitis + bile duct stone) and in ten out of 95 patients (10.5%) with chronic cholelithiasis. Although the growth rate may not be statistically significant, the results are higher ($p=0.063$).

Bacterial growth was detected among patients who underwent surgery due to cholelithiasis (10 patients, 66.7%), common bile duct stone with acute cholecystitis (3 patients, 20%) and acute cholecystitis (2 patients, 13.3%) as seen in Table 1.

In our study, the most common co-morbid diseases were diabetes mellitus (DM) and hypertension (HT) (six patients) among patients with positive bile cultures. Other diseases were heart disease, chronic obstructive pulmonary disease (COPD), obesity and goiter. However, nine out of 38 patients with concomitant diseases (23.6%) had positive bile cultures. Six out of 76 patients without concomitant diseases (7.8%) had positive bile cultures ($p=0.019$).

The affect of gender differences on bacterial cultures of bile ($p>0.05$) were not statistically significant.

Five of the 114 patients included in the study had complications in the early postoperative period. Complications included intra-abdominal bleeding in one patient, fluid accumulation in the surgical area in three patients and a surgical site infection in one patient. The patient who had bleeding was taken back to surgery and hemostasis was performed. The patients who had a serious accumulation of fluid were tracked and they recovered without additional treatment. The culture results of the other patients did not

indicate a need for additional antibiotic use. None of the patients with a positive culture had a surgical site infection in the early period. In our study, an infectious complication was seen in only one patient; no surgical site infections were observed in patients with positive bile cultures.

Additional diseases were detected in 38 of the 114 patients in the study. In 9 out of 15 patients with a positive bile culture concomitant diseases were identified. The most commonly seen diseases were hypertension and diabetes mellitus. Several concomitant diseases were seen along with a few co-morbidity diseases in some patients (Table 3).

DISCUSSION

Although normal people do not have bacteria in the gallbladder, it has been shown that bacteria exist in individuals with gallbladder stones³. After a cholecystectomy, these bacteria can cause infectious complications, especially when the gallbladder and bile ducts open and bile flows into the peritoneal cavity. Prophylactic use of antibiotics is used to prevent these infectious complications.

In different studies, the bacterial growth in the bile culture was found at different rates such as van Leeuwen PA et al. 16%⁵, Al Harbi M et al. 28%⁶, Abeyesuriya et al. 54%⁷, Mahafzah AM et al 20%⁸, Ohdan H et al. 38%⁹, den Hoed PT et al. 22%¹⁰, Samy AK et al. 19%¹¹. In our study, bacterial growth was seen in the bile culture of 15 of 114 patients (13.1%).

There are certain factors that increase bacterial growth in bile culture: acute cholecystitis, the obstruction of bile duct, diabetes mellitus, immunodeficiency, age over than 60 and ERCP^{5,9,11,12}. In this study, we evaluated the effects of these factors on growth.

Our literature review confirmed that patients over the age of 60 who had a cholecystectomy are more likely to have bacterial growth in the bile culture^{9,12}. In our study, we also identified significant bacterial growth in bile cultures of those older than 60. There were no statistically

significant differences between patients over and under the age of 60 when analyzed against the incidence of complications.

In one study¹², it has been determined that obstruction of bile tract or bile stasis causes bacterial growth in bile culture. The presence of growth in the bile cultures of three of our patients with acute cholecystitis and common bile duct stone supported this finding, but we did not find the rate of bile presence in the cultures of acute cholecystitis patients to be statistically significant.

According to our results, in the presence of comorbid disease an increase in bacterial growth in the bile culture may be expected. This result can be considered to be an effective factor in the presence of bacteria in the bile cultures.

The most frequently isolated bacteria found in the previous studies were *E. coli* and types of *Enterococ*¹⁴. In our study, the most commonly isolated bacteria are *Enterococ* spp., *E. coli* and *Enterobacter* spp. In the literature, it has been shown that more than one bacterium can grow in bile cultures at the same time¹¹. In our study, however, multi-bacterial growth was not observed.

A dose of 1 gram of Cefazolin was given intravenously to patients as a prophylactic prior to surgery. It was expected that the immediate administration of prophylactic antibiotics does not affect the bacterial flora of bile. One patient had a

postoperative surgical site infection. The prophylaxis is considered to be sufficient because there have not been any surgical site infections even in patients with positive bile cultures. In the literature, it has been reported that infectious complications after laparoscopic cholecystectomy are rarely seen⁷. Therefore, the use of prophylactic antibiotics was reopened for debate^{7,9}. It was seen that more bacteria grew in bile cultures of patients over 60 years of age with additional diseases and obstruction of bile.

In the literature, some studies suggest that there is a connection between surgical site infection and growth in the bile culture⁹, but other studies concluded otherwise^{6,10,13}. Our study did not include any patients with both surgical site infections and positive cultures.

CONCLUSION

In gallbladder pathologies, growth of bacteria occurring in the gallbladder can be enhanced by certain factors. These factors include age (over 60), concomitant diseases and the presence of bile flow obstruction. The risk of surgical site infection is lower in cholecystectomy wounds. However in our study, despite the presence of risk factors, antibiotic prophylaxis succeeded in preventing surgical site infections.

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