



The role of microbes and parasites in recurrent pyogenic cholangitis

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ABSTRACT

Objective: Recurrent pyogenic cholangitis (RPC) and ascariasis are prevalent in eastern India. Exact pathogenesis of RPC is still a matter of controversy. Hepatobiliary *Ascaris* infestation has been considered one of the causative factors in Eastern Asia, but conclusive evidence from India is lacking. RPC is associated with multi-drug-resistant (MDR) bacteria, which is a significant source of morbidity and mortality. This study aimed to assess the role of hepatobiliary ascariasis in pathogenesis of RPC and to study microbial profile and their implications in managing RPC patients.

Material and Methods: Consecutive patients with biliary stones who underwent surgery between March 2020 and December 2021 in a tertiary centre in eastern India were divided into RPC and non-RPC groups. Surgically retrieved samples of bile and biliary stones were sent for bacterial culture and microscopic and histopathological examination to identify the evidence of ascariasis in both groups and to study the microbial profile in RPC group.

Results: Eight out of 54 patients (14.8%) in the RPC group had evidence of hepatobiliary ascariasis. None of the patients in non-RPC group showed evidence of ascariasis. *Klebsiella* was the most common bacterial pathogen on bile culture, and 79% of bacterial isolates were MDR pathogens. Carbapenem group of antibiotics showed the highest sensitivity (66.6%) against bacterial culture growth in RPC patients.

Conclusion: This study showed a higher prevalence of hepatobiliary ascariasis in patients with RPC, but a conclusive etiological role is still lacking. RPC is associated with high incidence of MDR bacteria. Carbapenems may be considered the empirical antibiotic of choice in RPC.

Keywords: Ascariasis, cholangitis, multidrug resistance

INTRODUCTION

Recurrent pyogenic cholangitis (RPC) is characterised by repeated infections of the biliary system with the formation of stones and strictures. The exact pathogenesis of RPC is still a matter of controversy. Chronic bacterial infection and helminthic infestation remain the most likely possibilities. *Clonorchis sinensis* (CS) and *Ascaris lumbricoides* (AL) are the most commonly associated parasites with RPC (1). A study from Hong Kong has suggested the role of CS (2). AL is highly endemic in the Indian subcontinent, whereas CS is uncommon. Few studies indicate that parasites, their fragments, or ova can act as a physical nidus or nucleus, around which bile components can crystallise and form stones (3). Another perspective argues that the observed association between parasites and choledocholithiasis might be coincidental, especially in areas with high parasite prevalence (4). The geographic distribution of parasites and hepatobiliary stones might be relevant, as certain regions have a higher prevalence of both (3). Only a few studies from India have explored the etiological role of AL in RPC (5,6). This study aimed to identify the role of hepatobiliary ascariasis in the pathogenesis of RPC in Eastern India.

MATERIAL and METHODS

This prospective study included consecutive patients with hepatobiliary stones due to RPC and other aetiologies who underwent surgery between March 2020 and December 2021 in a tertiary care centre in Eastern India. The Institutional Ethics Committee approved this study. Written informed consent was obtained from all participants. The diagnosis of RPC was based on the clinical history of recurrent cholangitis, characteristic abnormalities on imaging, and soft brown pigment stones in intrahepatic ducts with or without stones in the common bile duct or gall bladder. Positive evidence of persisting or past hepatobiliary ascariasis was based on the identification of adult ascariasis on imaging or their recovery at surgery, histological examination showing worm fragments or ova forming the

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nidus of surgically retrieved hepatobiliary stones or bile specimen on light microscopy containing ova of AL. Patients with brown pigment stones in the intrahepatic or extrahepatic bile duct due to causes other than RPC were included in the non-RPC group. Patients with only gallbladder stones or cholesterol stones were excluded from the study. All recent and past medical records were gathered and analysed for evidence of recent and past hepatobiliary ascariasis. Bile samples (5 mL) were collected from the intrahepatic or extrahepatic bile duct during surgery. Bile sample was sent to the department of microbiology on the same day for culture for aerobic organisms and sensitivity against antibiotics. Multi-drug-resistant (MDR) was defined as antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories (7). Bile was centrifuged and examined under light microscopy for ova of AL by a team of microbiologists. Stones were retrieved from the extrahepatic or intrahepatic biliary tree at the time of surgery or ex vivo from the resected liver specimen. A senior operating surgeon visually inspected the stones to confirm their pigmented nature. Stones removed intact were sent to the pathology department in a clean, sterile container with 10% formalin solution. Stones were split into two to analyse the appearance and structure of the nucleus. Stones were treated with ethylenediamine tetraacetic acid (EDTA) solution to decalcify stones. Serial sections were cut and stained with hematoxylin and eosin. Multiple sections were examined for worm ova or worm fragments.

Statistics

Continuous data were reported as medians with interquartile ranges or as mean with standard deviation (SD). Categorical data were shown as frequency and percentages. Differences between categorical data were analysed using Fisher’s exact or Chi-square test. P< 0.05 was considered statistically significant. All analyses were performed using IBM SPSS, version 20 (IBM Corporation, Chicago, United States of America).

RESULTS

This study included 54 RPC patients and 39 non-RPC patients. In the RPC group, there were 16 men and 38 women with a mean age of 39.5 ± 12.01 years. The initial presenting features of the patients with RPC are shown in Table 1. Median duration of the illness was 30 months. A total of 24 patients

Table 1. Clinical presentation in RPC patients	
	n= 54 (%)
Abdominal pain	54 (100%)
Acute cholangitis	38 (70.3%)
Fever	35 (64.8%)
Jaundice	25 (46.2%)
Vomiting	17 (31.4%)

(44.4%) had undergone previous biliary surgery. A total of 24 patients (44.4%) had undergone previous Endoscopic retrograde cholangiopancreatography (ERCP), and three patients (5.6%) had undergone percutaneous radiological intervention for liver abscess in the past. Overall, 38 patients (70.4%) had undergone some biliary intervention in the past. Disease distribution was bilobar in 55.6% of the patients. The disease was confined to the left liver lobe in 37% of the patients (Figure 1). Only 7.4% of the patients had isolated right lobe RPC (Table 2). Liver resection was performed in a total of 30 patients (55.5%) (Table 3). Histological results were available in all RPC patients who underwent hepatectomy. All of them showed features of RPC.

Microbial Profile of RPC Patients

Bile was analysed for aerobic bacterial culture in 44 of 54 RPC patients. Culture results were positive in 33 patients (75%). The most common bacteria isolated on culture was *Klebsiella* in 42.4% of patients, followed by *Escherichia coli* in 39.3%. Types of organisms are shown in Table 4. In patients with a positive culture, MDR bacteria were found in 78.7%. Bacterial growths were most susceptible to carbapenem groups of antibiotics in 66.6%, followed by colistin in 63.6%. The lowest sensitivity was observed against the quinolones (27%) and cephalosporin (27%) group of antibiotics.

Etiological Evidence of Ascaris Lumbricoides

The primary indication of surgery in non-RPC patients is shown in Table 5. Biliary stones retrieved from RPC and non-RPC patients were brown pigment stones in all cases (Figure 2). Bile was analysed for the evidence of ascariasis in 36 patients in the RPC group and 32 patients in the non-RPC group. Unfertilised AL ova was found in two patients in the RPC group (Figure 3). On bile examination, no evidence of AL ova was seen in the non-RPC group. None of the patients in the RPC or non-RPC groups had evidence of AL on pathological examination of stones. Five patients in the RPC group had evidence of dead AL

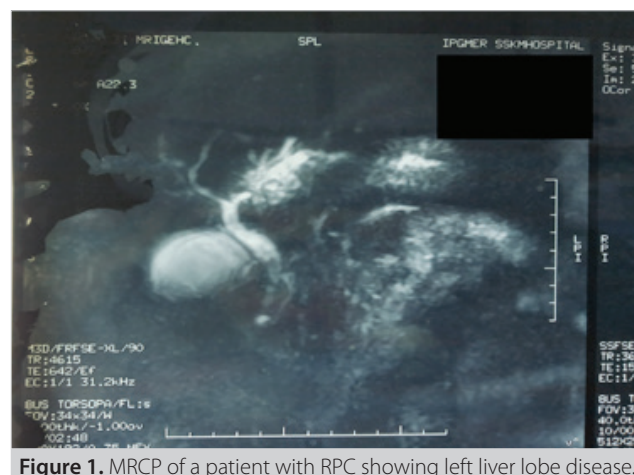


Figure 1. MRCP of a patient with RPC showing left liver lobe disease.

Table 2. Demographic data of RPC patients

	n= 54 (%)
Sex	
Male	16 (26.9%)
Female	38 (70.4%)
Age (years)	39.5 (21-62)
Duration of illness (months)	30 (2-264)
Previous hepatobiliary interventions	
Previous biliary surgery	24 (44.4%)
Cholecystectomy	16
CBD exploration	8
Previous ERCP and biliary stenting	24 (44.4%)
Previous image-guided percutaneous catheter drainage of liver abscess	3 (5.6%)
Disease distribution in liver	
Bilobar	30 (55.6%)
Left lobe	20 (37%)
Right lobe	4 (7.4%)

CBD: Common bile duct, ERCP: Endoscopic retrograde cholangiopancreatography.

Table 3. Types of surgery performed in RPC patients

Surgery	n= 54 (%)
HJ	24 (44.54%)
Left hepatectomy	8 (14.8%)
Left lateral sectionectomy	3 (5.6%)
Right hepatectomy	1 (1.9%)
Right posterior sectionectomy	1 (1.9%)
Left hepatectomy + HJ	4 (7.4%)
Left lateral sectionectomy + HJ	5 (9.3%)
Right hepatectomy + HJ	4 (7.4%)
Left hepatectomy + CBD exploration + T-Tube drainage	2 (3.7%)
Left lateral sectionectomy + CBD exploration + T-Tube drainage	2 (3.7%)

HJ: Hepaticojunostomy, CBD: Common bile duct.

on review of previous imaging. Out of five patients, two patients had evidence of AL exclusively on abdominal ultrasound, one patient exclusively on MRCP, and two patients on both abdominal ultrasound and MRCP (Figure 4,5). One patient in the RPC group was found to have a history of biliary ascariasis 25 years ago based on a written clinical record. In contrast, none of the patients in the non-RPC group had a history of hepatobiliary ascariasis. Overall, 8 (14.8%) patients in the RPC group had evidence of hepatobiliary ascariasis, while no patient had evidence of biliary ascariasis in the non-RPC group ($p= 0.019$) (Table 6).

DISCUSSION

Management of RPC requires a multidisciplinary team approach. Basic principles of treatment include (i) complete removal of all

stones, (ii) resecting the non-functioning segments of liver, and (iii) establishing adequate biliary drainage. Both operative and non-operative interventions are described for the management of RPC. Non-operative procedures include percutaneous transhepatic cholangioscopic lithotripsy and, endoscopic retrograde cholangiopancreatography (ERCP) and stone extraction (8,9). Surgical treatment of RPC generally gives better results than non-operative techniques (10). RPC involving the first-order bile ducts is usually treated by biliary enteric bypass procedures. Whereas, associated liver abscesses, liver atrophy, third-order ductal stones, and intrahepatic cholangiocarcinoma are best treated with liver resection. In the current study, left sided liver involvement was more common (93%) and isolated right-sided involvement was only (7%), which is in concordance

Table 4. Microbial profile of bile culture in RPC patients

Bacterial growth present	33 (75%)
Types of bacteria	
<i>Klebsiella</i>	14 (32%)
<i>E. coli</i>	13 (30%)
<i>Acinetobacter baumannii</i>	5 (11%)
<i>Staphylococcus</i>	1 (2%)
MDR	26 (79%)
Sensitivity to antibiotics	
Carbapenem*	22 (67%)
Colistin	21 (64%)
Aminoglycoside#	19 (57.5%)
Polymyxin	15 (45%)
Penicillins [§]	10 (30%)
Quinolones**	9 (27%)
Cephalosporins***	9 (27%)

MDR: Multidrug resistant.
 *Carbapenem includes meropenem, imipenem.
 **Quinolones include ciprofloxacin, levofloxacin.
 ***Cephalosporin include ceftriaxone, cefepime, ceftazidime.
 §Penicillins include amoxicillin, piperacillin.
 #Aminoglycosides includes amikacin, gentamycin.

Table 5. Indication of surgery in non-RPC patients

Diagnosis	n= 39
Choledocholithiasis	18
Choledochal cyst with cystolithiasis	14
Hepaticojejunostomy site stricture with hepatolithiasis	5
Post-cholecystectomy biliary stricture with hepatolithiasis	2



Figure 2. Multiple brown pigment stones with intrahepatic biliary ductal dilatations are seen in the left lobe of the liver.

with recent studies (11,12). Left hepatectomy was the most common resection procedure performed in the present study (26%), which is in concordance with the recent study (13).

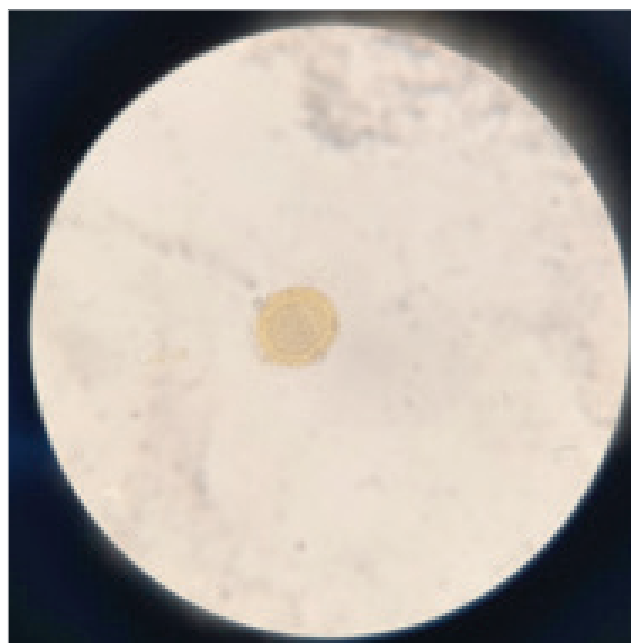


Figure 3. Unfertilised *Ascaris* ova is seen in bile on light microscopy in the RPC patient.



Figure 4. Ultrasound of the abdomen showed linear hyperechoic density in CBD (arrow), which suggests dead AL.

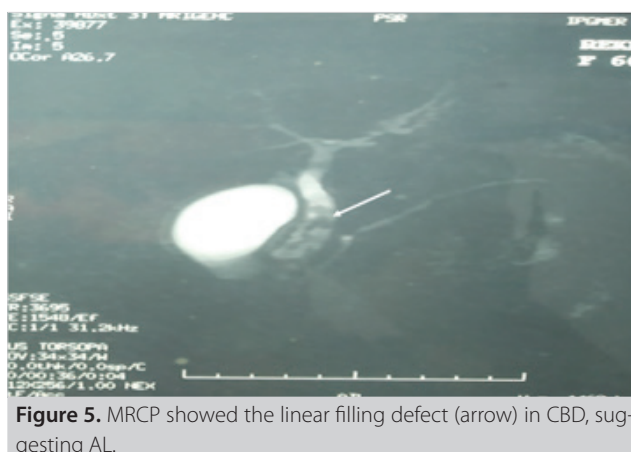


Figure 5. MRCP showed the linear filling defect (arrow) in CBD, suggesting AL.

Table 6. Evidence of hepatobiliary ascariasis in RPC and non-RPC groups

AL Detection Method	RPC (n= 54)	non-RPC (n= 39)	p
Evidence of hepatobiliary ascariasis in imaging	5	0	0.071
Ultrasonography only	2	0	
MRCP only	1	0	
Combined ultrasonography and MRCP	2	0	
Documented history of previous hepatobiliary ascariasis	1	0	1.000
Stone analysis	45	34	1.000
Stone positive for AL	0	0	
Bile analysis	36	32	0.494
Bile positive for AL	2	0	
Overall evidence for ascariasis	8	0	0.019

Causative factors described in the development of the RPC disease include primary bacterial infection of bile ducts, parasitic infections, and a diet containing high carbohydrates, low protein, and low saturated fat (14,15). Khuroo et al. have reported the only etiological study on RPC from northern India, and the authors have demonstrated the evidence of hepatobiliary ascariasis in 24 out of 30 patients with RPC in which the authors have compared the RPC group with a group of 30 patients with gallstones and found only one patient had evidence of AL (6). Twenty-two out of 30 patients had evidence of AL on pathological examination of biliary stones, three patients had positive evidence on microscopy of bile, and five patients had a history of hepatobiliary ascariasis. The authors suggested that fragments of dead worms or ova form nidus of brown pigment stones in RPC and play a key role in initiating subsequent bacterial infection and stricture formation. Similar conclusions have also been reported in studies from China and the Philippines (16,17). In the present study, we may divide the evidence into hard evidence, such as parasite fragment or egg as stone nidus and soft evidence, including the presence of ova in bile or the presence of radiological presence of hepatobiliary ascariasis. The presence of parasite fragments or ova in stone is logically the most robust evidence to prove the etiological role of AL in disease pathogenesis; however, in the present study, the stone analysis failed to demonstrate the AL ova or fragment as a nidus for stone formation. Two possible explanations may contribute to the variable detection of parasitic infection in patients with RPC across the studies. First, inter-study variations in diagnostic methodologies, with inherent differences in sensitivity, contribute to challenges in establishing a definitive prevalence for ascariasis. Second, the infection may have resolved after initiating the disease without leaving detectable evidence of the inciting cause. In support of the latter hypothesis, there are studies in which the analysis of stones and bile have shown proof of ascariasis, such as debris and ova, which may have served as a nidus for stone formation and biliary strictures (6,14,16). In the present study, MDR isolates

were found in 78.7% of the patients with positive culture in RPC patients, which is significantly higher than the reported incidence of 20-30% in acute cholangitis (17). The development of MDR biliary pathogens in cholangitis has been attributed to previous antibiotic use within 90 days and previous biliary intervention, among other factors (18). In the present study, the high incidence of MDR pathogens can be attributed to previous biliary intervention in the majority of patients, including biliary stenting and biliary surgery, due to repeated use of antibiotics for previous cholangitis episodes, empirical use of antibiotics when bile culture is not possible or available and frequent change of antibiotic group to treat cholangitis empirically. MDR pathogens have been attributed to adverse surgical outcomes in RPC patients (19,20). In cases of acute cholangitis, effective treatment of bile duct obstruction is paramount. Regarding the choice of antibiotics in community-acquired cholangitis, the guidelines are based on 3rd-generation cephalosporin, associated with an anaerobic agent in cases involving biliary enteric anastomosis (19,21). However, given the high rates of resistance in RPC, a different approach to antibiotic management may be required. In the present study, the carbapenem group showed the highest sensitivity (66.6%) against bacterial culture growth, followed by colistin (63.6%), while quinolones (27%) and cephalosporin groups showed the lowest sensitivity (27%). Carbapenems may be considered empirical antibiotics of choice in RPC patients with severe cholangitis and require an aggressive treatment approach when the bacteria culture and antibiotic sensitivity pattern are unavailable. Cephalosporin and quinolone groups of antibiotics should be avoided to empirically treat severe cholangitis in RPC before culture reports become available. Finally, antimicrobial therapy must be secondarily adapted to bacteriological test results to reduce the risk of emergent MDR pathogens. This is one of the few studies from India and the first from Eastern India evaluating the role of AL in RPC. The prevalence of RPC is high in Eastern India compared to other parts of the country; therefore, further studies with a much

larger sample size are required to identify the exact aetiology of this region-specific disease.

CONCLUSION

The present study showed a higher prevalence of hepatobiliary ascariasis in patients with RPC. However, the exact mechanism of ascariasis in forming biliary stones or initiating the disease is still unclear. RPC is a disease with a high incidence of MDR pathogens; therefore, judicious use of antibiotics is required during treatment. Carbapenems may be considered the empirical antibiotic of choice in RPC patients with severe cholangitis.

Ethics Committee Approval: This study was obtained from Institute of Postgraduate Medical Education and Research Ethics Committee (Decision no: IPGME&IEC/2020/221, Date: 11.03.2020).

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Author Contributions: Concept – SR; Design – SR, ZA; Supervision – All of authors; Data Collection and/or Processing – ZA; Analysis and/or Interpretation – ZA; Literature Search – All of authors; Writing Manuscript – ZA; Critical Reviews – ZA.

Conflict of Interest: The authors have no conflicts of interest to declare.

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**ORİJİNAL ÇALIŞMA-ÖZET**

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Tekrarlayan piyojenik kolanjitte mikrop ve parazitlerin rolüZuber Ansari¹, Sukanta Ray², Somak Das², Tuhin Subhra Mandal²¹ FH Tıp Fakültesi ve Hastanesi, Genel Cerrahi Anabilim Dalı, Cerrahi Gastroenteroloji Bilim Dalı, Agra, Hindistan² Lisansüstü Tıp Eğitimi ve Araştırma Enstitüsü, Cerrahi Gastroenteroloji Anabilim Dalı, Kolkata, Hindistan**ÖZET**

Giriş ve Amaç: Tekrarlayan piyojenik kolanjit (TPK) ve askariyazis Hindistan'ın doğusunda yaygındır. TPK'nin kesin patogenezi hala tartışma konusudur. Hepatobiliyer Askaris enfestasyonu Doğu Asya'da TPK'nin nedenlerinden biri olarak kabul edilmiştir, ancak Hindistan'dan bu konuda kesin kanıtlar eksiktir. TPK, önemli bir morbidite ve mortalite kaynağı olan çoklu ilaca dirençli (ÇİD) bakterilerle ilişkilidir. Bu çalışmanın amacı, hepatobiliyer askariyazisin TPK patogenezindeki rolünü değerlendirmek ve mikrobiyal profili ile bunların TPK hastalarının yönetimindeki etkilerini incelemektir.

Gereç ve Yöntem: Hindistan'ın doğusunda bulunan üçüncü basamak bir merkezde, Mart 2020-Aralık 2021 tarihleri arasında ardışık ameliyat edilen safra taşı hastaları TPK olan ve TPK olmayan olarak iki gruba ayrıldı. Cerrahi olarak alınan safra ve safra taşı örnekleri, her iki grupta askariyazis kanıtlarını belirlemek ve TPK grubundaki mikrobiyal profili incelemek için bakteri kültürü, mikroskopik ve histopatolojik inceleme yapılmak üzere gönderildi.

Bulgular: TPK grubundaki 54 hastanın sekizinde (%14,8) hepatobiliyer askariyazis bulguları tespit edildi. TPK olmayan gruptaki hastaların hiçbirinde askariyazis bulgusuna rastlanmadı. *Klebsiella*, safra kültüründe en sık görülen bakteriyel patojeni ve bakteriyel izolatların %79'u çoklu ilaca dirençli (ÇİD) patojenlerdi. Karbapenem grubu antibiyotikler, TPK hastalarında bakteri kültürü üremesine karşı en yüksek duyarlılığı (%66,6) gösterdi.

Sonuç: Bu çalışma, TPK'li hastalarda hepatobiliyer askariyazis prevalansının daha yüksek olduğunu göstermiştir, ancak kesin bir kanıt hala eksiktir. TPK, yüksek ÇİD bakteri insidansı ile ilişkilidir. Karbapenemler TPK'de tercih edilen ampirik antibiyotik olarak düşünülebilir.

Anahtar Kelimeler: Askariyazis, kolanjit, çoklu ilaç direnci

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