Biliary ascariasis in the etiology of recurrent pyogenic cholangitis in an endemic area

Mohammad S. Khuroo, Naira S. Khuroo, Mehnaaz S. Khuroo

ABSTRACT

Aims: The role of biliary ascariasis in etiology of recurrent pyogenic cholangitis in endemic areas has not been well studied. Methods: We studied 30 patients of recurrent pyogenic cholangitis with brown pigment stones and 30 patients with cholesterol gallstones. Stones from 22 patients (11 brown pigment stones and 11 gallstones) were examined by infrared spectrophotometry to substantiate the classification of stones done on visual inspection. Biliary calculi were considered to be associated with *Ascaris lumbricoides* in those with: previous documented evidence of biliary ascariasis, bile specimens containing ova of *Ascaris lumbricoides* on light microscopy and worms, worm fragments or their ova forming the nidus of stones on histological examination. Results: Bile cultures grew microorganisms in 24 (80%) patients with recurrent pyogenic cholangitis while only one patient with gallstones grew *Escherichia coli* from bile. None of the patients in either group had past or persisting evidence of clonorchiasis. Five patients had previous documentation of biliary ascariasis. In three patients bile contained ova of *Ascaris lumbricoides* and stones in 22 patients contained worm, worm fragments or their ova on histological examination. Overall 24 (80%) patients with recurrent pyogenic cholangitis had past or persistent evidence of biliary ascariasis while only one patient with gallstones had worm fragments on histopathology of stones (p < 0.001). Conclusion: We conclude that in endemic areas bacterial infection of the bile ducts in recurrent pyogenic cholangitis occurs with biliary invasion by *Ascaris lumbricoides* and dead worms, their fragments or ova form nidus of brown pigment stones formation in such patients.

Keywords: *Ascaris lumbricoides*, Biliary ascariasis, Recurrent pyogenic cholangitis

INTRODUCTION

Recurrent pyogenic cholangitis (RPC) is a disease characterized by chronic infection and stone formation in the bile ducts [1]. It mainly affects inhabitants of Southeast Asia and is most prevalent in Taiwan and the south of China. In Hong Kong, it is most common disease...
of the biliary tract and the third most frequent cause of acute abdominal pain requiring surgical intervention. The disease has also been reported from Indonesia, Japan, Singapore, Malaysia, South Africa and Chinese Immigrants in Canada [2–9]. In India (Kashmir), RPC constitutes 12.5% of all patients with biliary disease [10]. This unusual biliary tract syndrome is now being encountered with increased frequency in Western societies especially in the United States, largely as a result of increased immigration of refugees from Asia during and after the Vietnam War [11–13].

The clinical presentation of patients with RPC is characteristic. Majority of patients present with recurrent right upper quadrant pain, nausea and vomiting, often accompanied by fever, shaking chills and jaundice [2]. The intrahepatic and extrahepatic ducts are dilated and contain soft brown pigment stones, biliary mud and/or pus. The gallbladder is involved in only 15% of patients. As a result of recurrent cholangitis and stone formation, bile ducts develop strictures, excessive branching and arrowhead formation of the intrahepatic branches [14]. Bile cultures invariably yield Escherichia coli, or other colonic aerobic flora [2, 10, 11]. Secondary changes in liver biopsy are characteristic of obstruction and cholangitis [15]. Portions of the liver may atrophy as a result of long standing obstruction. If infection gets the upper hand, intrahepatic abscess formation and septicaemia may supervene [2].

Although RPC has been recognized for over half a century, its exact etiopathogenesis has remained unknown [16]. The major controversy is on the route of infection into the bile ducts. The popular hypothesis include: a) portal bacteraemia leading to portal phlebitis which secondarily spreads to ductal system [15]; (b) invasion of bile ducts by parasites, viz. Ascaris lumbricoides and Clonorchis sinensis, which carry enteric organisms along with into the bile ducts [4, 11, 17, 18]. Ascaris lumbricoides is highly endemic and Clonorchis sinensis infection is not known to occur in Kashmir, India. This made us to suspect an association between biliary ascariasis and recurrent pyogenic cholangitis. In this study, we looked for evidence of past or persisting biliary invasion by Ascaris lumbricoides in patients with RPC and compared it with those of cholesterol gallstones.

MATERIALS AND METHODS

The study group included 30 consecutive patients with RPC. 30 patients with cholesterol gallstones, age and sex matched with the study group, formed the control group. Diagnostic Criteria. The diagnosis of RPC was made on the basis of the following criteria [14, 19]:

(a) recurrent (more than 3 attacks) of right upper abdominal pain, fever, rigors and jaundice
(b) elevated serum alkaline phosphatase levels more than twice the upper limit of normal (normal values 1.63–4.65 microkat/l)
(c) cholangiographic abnormalities viz. dilatation, strictures, excessive branching and arrowhead formation of intrahepatic bile ducts;
(d) soft brown pigment stones in the hepatic ducts and/or common bile duct, but selectively sparing the gallbladder;
(e) stones containing calcium bilirubinate as a principal component [20].

The following groups of patients with cholangitis were excluded from the study:

(a) patients with cholesterolithiasis with choledocholithiasis
(b) patients with choledocholithiasis following previous cholecystectomy
(c) patients with bile duct strictures or injury after previous biliary surgery
(d) patients with biliary and pancreatic neoplasm
(e) patients with biliary ascariasis or hydatidosis
(f) patients who were found to have cholesterol or black pigment stones at surgical exploration or endoscopic sphincterotomy.

Patients were diagnosed to have cholesterol gall stones on the basis of the following criteria [16, 21]: (a) stones located in gallbladder; (b) on visual inspection stones of cholesterol type. The following criteria were considered as evidence of past or persisting Ascaris invasion of the biliary tree:

(a) delineation of adult Ascaris in the biliary tree by imaging techniques or their recovery at surgery done more than two years prior to the diagnosis of RPC;
(b) bile specimen on light microscopy containing ova of Ascaris lumbricoides;
(c) histological examination showing worm, worm fragments or ova forming the nidus of stones.

Bile specimens were collected at laparotomy by direct puncture of common bile duct or by a cannula passed into common bile duct through the cystic duct. Bile was cultured for aerobic and anaerobic organisms by established methods [22]. An aliquot of bile was centrifuged and examined under light microscopy for ova of Ascaris lumbricoides and Clonorchis sinensis [2]. Each stone obtained at laparotomy was washed with deionized distilled water until supernatant became clear, was split carefully into two so as not to damage the broken surface; and its appearance and structure of the nucleus, interior and surface areas were inspected [23]. On visual inspection, gallstones were classified as cholesterol stones, brown pigment stones and black pigment stones on the basis of criteria reported previously [24].

Afterwards, stones were sliced with a knife and fixed in 10% formal saline, dehydrated, cleared and embedded in paraffin wax. Serial sections were cut and stained with hematoxylin and eosin, PAS-Alcian blue pH 2.5, Masson’s trichrome and orcein. Multiple sections were examined for worm or worm fragments and classified as adult worms, cuticle and ova. Ascaris lumbricoides and Clonorchis sinensis were recognized on the basis of
characteristic worm appearance, cuticular morphology and ova characteristics [25].

Stones from 22 patients (11 brown pigment stones and 11 cholesterol stones) were examined by chemical microscopy, infrared spectrophotometry, polarized light microscopy and X-ray diffraction as reported earlier [20]. The data on various stone components were included in this study to further substantiate the classification of stones done on visual inspection.

Endoscopic retrograde-cholangiopancreatography (ERCP) was obtained using JFIT side viewing duodenoscope. Cholangiograms were read by two experienced personal without knowledge of the clinical details. Cholangiographic abnormalities were reported as described elsewhere [14].

The diagnosis of biliary ascariasis was made on characteristic sonographic and cholangiographic appearance as described earlier [26–28].

The study was approved by the ethical committees of Sher-e-Kashmir Institute of Medical Sciences and Dr. Khuroo’s Medical Clinic and all the patients gave a written informed consent for the study. The values were expressed as mean ±1 SD. The two groups were compared by X² with Yates correction.

RESULTS

Study group: Thirty patients (10 men and 20 women) with a mean age of 35.9±15.2 years constituted the study group. The mean duration of disease was 4.1±4.2 years (range 11 months to 20 years) and all had recurrent episodes of right upper quadrant pain, fever with chills and jaundice. Stool analysis revealed ova of Ascaris lumbricoides in all patients; however, none of the patients had ova of Clonorchis sinensis. Liver function tests revealed mean serum bilirubin levels of 116.2±152.1 micro mol/L, serum alkaline phosphatase 18.65±12.18 microlat/L and mean serum alanine aminotransferase level of 1.66±1.25 micro kat/l. In 24 patients bile samples grew organisms on culture which included Escherichia coli in 12, Klebsiella aerogenes in 10 and in Streptococcus faecalis 2 patients. Stones were located in the hepatic ducts in seven patients, hepatic ducts and common bile duct in 12 patients and in common bile duct alone in 10 patients. None of these patients had stones inside the gallbladder. Cholangiogram revealed biliary dilatation in all patients, excessive branching of hepatic ducts in 15 (50%) patients, strictures of common bile duct and/or hepatic ducts in 16 (53%) patients and abrupt termination and arrowhead formation of intrahepatic ducts in 12 (40%) patients. All patients had surgery, cholecchoctomy and stone extraction form bile and hepatic ducts. Five patients with disease of the left lobe had in addition left hepatic lobectomy. At laparotomy brown pigment stones were recovered from bile ducts in all the patients. The number of stones recovered from each patient varied from 1–10 (mean ±SD 7±3) and the size of stones varied from 0.5 cm to 2.5 cm (mean 1.1±0.9 cm). On cut sections, the stones had a characteristic laminated appearance due to brownish yellow and granular pigmented material.

Control group. Thirty patients with gallstones constituted the control group which included 10 men and 20 women with mean age of 38.8±8.4 years and the mean duration of disease from 11.7±17.9 months (range 1 month to 8 years). Of these, 27 patients presented with recurrent biliary colic, two patients had suffered from acute cholecystitis and one patient had an episode of acute cholangitis. Stool analysis revealed ova of Ascaris lumbricoides in five patients but ova of Clonorchis sinensis were not seen in any of them. Liver function tests did not reveal any abnormality in serum bilirubin, alkaline phosphatase or alanine aminotransferase. All the 30 patients had stones inside the gallbladder and one patient, in addition, had a stone in the common bile duct. Bile cultures were sterile in 29 patients while Escherichia coli were grown on culture in one patient. On visual inspection, the stones were of cholesterol type in all the 30 patients.

Biliary stone analysis was done in 22 patients including 11 patients each with recurrent pyogenic cholangitis and gallstones. Cholesterol formed the major component of gallstones while in brown pigment stones calcium bilirubinate was the major component (Table 1).

None of the patients in the study or control group had evidence of past or persisting infection of biliary tree by Clonorchis sinensis. 24 (80%) of the 30 patients in the study group had evidence of either past and/or persisting invasion of biliary tract by Ascaris lumbricoides. In contrast only one patient with gallstones on histological examination showed Ascaris cuticle forming the nidus of the stone (Table 2). Two patients with previous documentation of biliary ascariasis had formed stones which did not contain worm or worm fragments or ova on histological examination.

Five patients (two men and three women) had evidence of biliary ascariasis 5.3±5.8 years (range 3 to 16 years) prior to the diagnosis of recurrent pyogenic cholangitis and development of bile duct stones. One patient had four episodes of biliary invasion by Ascaris lumbricoides over an eight-year period. The diagnosis of biliary ascariasis was documented on sonography in one patient, ERCP in three patients (Figure 1), and at laparotomy in one patient.

Bile specimens on light microscopy showed ova of Ascaris in 3 patients. These ova were both fertilized and unfertilized and showed various stages of degeneration (Figure 2). These three patients had previous biliary invasion by Ascarids more than three years prior to the diagnosis of recurrent pyogenic cholangitis.

Of the 22 patients with worm, worm fragments formed the nidus of the stones, five patients had adult Ascarids, eight cuticle fragment and 9 ova of Ascaris lumbricoides in the stone sections (Figures 3 and 4).
DISCUSSION

The first National Institute of Health International workshop on pigment gallstones classified all pigment stones and brown pigment stones and it was agreed that pigment stones can be distinguished from cholesterol stones and between one another by gross inspection [15]. Brown pigment stones located in the intrahepatic and extrahepatic bile ducts are characteristic of recurrent pyogenic cholangitis and calcium bilirubinate is the principal component of such stones. The biliary stones in the study group in this report had all the characteristics of those reported in patients with recurrent pyogenic cholangitis from the Orient. This was in contrast to stones in the control group which were located in the gallbladder and cholesterol formed the principal constituent of these stones. Brown pigment stones are associated with bacterial infection of the bile ducts by Escherichia coli and other colonic aerobic flora. These organisms produce high amounts of beta glucuronidase which causes deconjugation of the bile pigments and their precipitation as stones [18, 22]. Low protein diet leads to lower activity of glucaro-1,4-lactone which is a natural inhibitor of beta glucuronidase and promotes stone formation in such patients [18].

Although the role of bile duct infection in the pathogenesis of brown pigment stones is well established, the route of infection into the biliary tree continues to be a matter of debate. The popular theory that portal bacteraemia causing portal phlebitis secondarily spreads to the ductal system is fraught with major criticism. The infection having reached into the liver is excreted...

Table 1: Data on stone analysis showing various components in 11 brown pigment stones and 11 gallstones

<table>
<thead>
<tr>
<th>No.</th>
<th>Component</th>
<th>Brown pigment stones(%± S.D)</th>
<th>Gall stones(± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol</td>
<td>21.27±13.75</td>
<td>92.25±4.0</td>
</tr>
<tr>
<td>2</td>
<td>Calcium Bilirubinate</td>
<td>62.7±19.2</td>
<td>4.25±2.1</td>
</tr>
<tr>
<td>3</td>
<td>Calcium Carbonate</td>
<td>-</td>
<td>0.8±1.3</td>
</tr>
<tr>
<td>4</td>
<td>Mixed bile pigments</td>
<td>15.9±12.2</td>
<td>1.6±1.7</td>
</tr>
<tr>
<td>5</td>
<td>Calcium salts of fatty acids</td>
<td>3.0±2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2: Data showing evidence of biliary invasion by Ascaris lumbricoides in study and control group

<table>
<thead>
<tr>
<th>Evidence of biliary invasion by Ascaris</th>
<th>Recurrent pyogenic cholangitis (n=30)</th>
<th>Gall stones (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous Hepatobiliary ascariasis</td>
<td>5*</td>
<td>0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Bile positive for ova of Ascaris lumbricoides</td>
<td>3</td>
<td>0</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Stones Positive for Ascaris lumbricoides</td>
<td>22</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Three patients had stones positive for Ascaris while in two patients, stones did not contain Ascaris on histological sections.

Figure 1: Recurrent pyogenic cholangitis. 30-year-old female with recurrent attacks of cholangitis for six months presented with severe pyogenic cholangitis and septic shock. She was admitted to intensive care unit and had emergency ERCP and endoscopic naso-biliary drainage. This patient was diagnosed with biliary ascariasis five years prior to this episode of cholangitis. (A) Showing ERCP Cholangiogram obtained 5 year prior to present admission revealing two long linear smooth filling defect in the common and left hepatic duct (arrows), (B) Showing ERCP cholangiogram performed during present admission. Cholangiogram revealed biliary dilatation with multiple filling defects and cholangitic changes in the common hepatic, right and left hepatic duct. Naso-biliary tube is in place to treat pyogenic cholangitis.

Figure 2: Microscopy of bile sample obtained from a patient with recurrent pyogenic cholangitis showing (A) fertilized, and (B) unfertilized ova of Ascaris lumbricoides.
into the bile and it is generally agreed that no serious consequences would ensue as long as there is free biliary drainage [4]. Bockus considers that portal bacteraemia is harmless under ordinary circumstances [29] and Wilkis also believes that bacteria excreted into the bile will not usually give rise to infection [30]. Moreover, comparative studies in the West and Orient have shown similar percentage of portal bacteraemia in surgical patients, yet recurrent pyogenic cholangitis has not been reported from western countries [2]. This hypothesis also fails to explain the special geographical distribution of recurrent pyogenic cholangitis.

Many authors have postulated association of recurrent pyogenic cholangitis with biliary infestation by \textit{Clonorchis sinensis} and/or \textit{Ascaris lumbricoides}. The hypothesis is based upon geographical distribution of recurrent pyogenic cholangitis which resembles that of clonorchiasis and ascariasis. About half of the patients with RPC are infected with \textit{Clonorchis sinensis} and about 12.9% have evidence of biliary ascariasis [14,16,19]. \textit{Ascaris lumbricoides} and \textit{Clonorchis sinensis} formed the nidus of the stones in 38% and 19% patients of RPC respectively in a study from Hong Kong [17]. In one study from South Africa on the role of ascariasis in non-western pattern of biliary stones, \textit{Ascaris lumbricoides} was delineated in the biliary tree in 14 of the 15 patients [9]. However, many authors have casted doubt on such an association and believe that RPC and biliary parasites are common in the regions of world with low socioeconomic status and that their co-existence in the same patient may be related to their high prevalence without any cause and effect relationship. Up till now, there is no case control study available in literature which studies the role of biliary parasites in a group of patients with brown pigment stones and a control group with different type of stones.

Our data clearly speak of a strong association between biliary invasion by \textit{Ascaris lumbricoides} and recurrent pyogenic cholangitis. In contrast, age and sex matched controls with cholesterol gallstones did not show such an association. In five patients biliary invasion by round worms preceded the diagnosis of RPC by many years. Previous data from Kashmir have shown that 12.6% of patients with biliary ascariasis when followed for long time form brown pigment stones in the hepatic ducts [10]. These data were strongly in favor of a causal relationship of biliary ascariasis in recurrent pyogenic cholangitis.

The pathogenesis of bile duct infection and stone formation and stone formation in patients of recurrent pyogenic cholangitis following biliary invasion by round worms is multifactorial. Once \textit{Ascaris lumbricoides} invades the biliary tree, it also carries along with enteric organisms. It also causes partial obstruction of the bile ducts leading to inadequate biliary drainage, bile stasis and establishment of infection in the biliary tree. Recently we have shown that recurrent \textit{Ascaris} invasion causes papillitis which induces motor abnormalities resulting in delayed biliary drainage and recurrent episodes of cholangitis [19]. Once the \textit{Ascaris} invade the biliary tree, they usually move out of the bile ducts as they are very agile and motile. Occasionally, however, they may get trapped and die inside the bile ducts. Worm
extracts contain high activity of beta glucuronidase which facilitates deconjugation of bile pigments. Dead worms, ova and worm fragments act as a potential nidus for stone formation. Data from the present study showed that 22 of the 30 stones analyzed contained worms, worm fragments or their ova as the nidus of stones. Maki in 1965 showed in vitro studies with remarkable precipitation of calcium bilirubinate on to the surface of Ascaris eggs that were incubated in human bile [18].

The present study was done in an endemic area of ascariasis. Clonorchis sinensis has not been reported from Kashmir. It is, however, prevalent in many countries of South East Asia from where RPC has been reported. In these countries, the association of Clonorchis sinensis with recurrent pyogenic cholangitis has been established. About half of the patients with RPC in these countries are infected with this parasites and about 16% of brown pigment stones in these patients contained ova of Clonorchis sinensis [17]. Clonorchis sinensis migrates from duodenum into the biliary tree where it takes from duodenum into the biliary tree where it takes

CONCLUSION

We conclude that in endemic areas bacterial infection of the bile ducts in recurrent pyogenic cholangitis occurs with biliary invasion by Ascaris lumbricoides and dead worms, their fragments or ova form nidus of brown pigment stones formation in such patients. However, our hypothesis of association between biliary ascariasis and RPC needs to be substantiated by exact mechanism of stone formation. The rate of worm infestation in our population is very high (26). The best form of prevention is to keep the gut free of worms and to prevent, reinfection by improving hygienic methods and regular three monthly courses of anthelmintic therapy. The clinicians should be aware of relationship between biliary ascariasis and RPC and apply this knowledge in routine clinical practice.

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Author Contributions

Khuroo Mohammad Sultan – Substantial contribution to concept and design, Acquisition and analysis of data and interpretation of data, Drafting of article, revising critically for intellectual content, Final approval of the version to be published

Khuroo Naira Sultan – Substantial contribution to concept, design, Acquisition of data, Drafting of article, Final approval of the version to be published

Khuroo Mehnaaz Sultan – Substantial contribution to concept and design, Analysis and interpretation of data, Drafting of article, revising critically for intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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**Article citation:** Khuroo MS, Khuroo NS, Khuroo MS. Biliary ascariasis in the etiology of recurrent pyogenic cholangitis in an endemic area. Int J Hepatobiliary Pancreat Dis 2015;5:22–29.

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