Clonorchiasis, which is caused by Clonorchis sinensis

1. Introduction

Diagnosis and Risk Factors

Clonorchiasis in Patients with Biliary and Pancreatic Diseases: Research Article

ff

globally whom live in China and other parts of East Asia [1–3]. An investigation conducted from 2005 to 2014 in Guangxi Province, southern China, showed that the prevalence of clonorchiasis in the general population reached 9.9% [4]. Clonorchiasis causes mechanical and chemical injury, resulting in inflammation [5–7], obstruction [5–8], and cancerogenesis [1, 2, 9] in the intrahepatic and extrahepatic biliary tracts [8, 10]. Common assays for diagnosing clonorchiasis include serologic detection of parasite-specific antibody and DNA [11–15], egg detection in bile and fecal samples [16, 17], and imaging [18]. The detection of C. sinensis eggs in fecal samples is specific, but with low sensitivity [19, 20], and requires skillful technique [21], especially when the infection is mild or there is biliary obstruction. Endoscopic retrograde cholangiopancreatography (ERCP) is not only a method for angiography but also a safe and effective tool for the treatment of biliary and pancreatic disorders. C. sinensis eggs are detectable in bile [6, 7, 19], but few studies have compared the detection of C. sinensis between bile and fecal samples.

It is important to evaluate independent risk factors for clonorchiasis in a large cohort of patients. Although several general risk factors for clonorchiasis have been reported [22, 23], no study to date has examined the risk factors associated with endoscopic findings. Various biliary or pancreatic disorders require different endoscopic interventions. ERCP procedures are generally safe and effective, but endoscopists must have a thorough understanding of indications for the

Research Article

Clonorchiasis in Patients with Biliary and Pancreatic Diseases: Diagnosis and Risk Factors

Guolin Liao,1 Huaqiang Ruan,1 Peng Peng,1 Shiquan Liu,1 Jianfu Qin,1 Zhihai Liang,2 Guodu Tang,2 Mengbin Qin,1 and Jie’an Huang1

1Department of Gastroenterology, The Second Affiliated Hospital of Guangxi Medical University, Nanning 530007, China
2Department of Gastroenterology, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, China

Correspondence should be addressed to Mengbin Qin; dr.mmbin@hotmail.com and Jie’an Huang; hjagxmu@163.com

Received 24 September 2019; Accepted 3 February 2020; Published 24 February 2020

Academic Editor: Michel Kahaleh

Copyright © 2020 Guolin Liao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Many epidemiological studies have investigated the risk factors for clonorchiasis, but endoscopic findings of this disease in endoscopic retrograde cholangiopancreatography (ERCP) have not been well characterized. In this study, we evaluated clonorchiasis in ERCP in patients with biliary and pancreatic diseases. Methods. This was a retrospective two-center study in hospitalized patients who received ERCP between January 2012 and October 2018. All patients were divided into clonorchiasis and nonclonorchiasis groups. Data were analyzed using univariate analysis and multivariate analyses. Results. A total of 1119 patients were included, and clonorchiasis was diagnosed in 19.2% patients. Detection of C. sinensis eggs in bile samples was higher than that in fecal samples (85.9% vs. 58.7%; P = 0.001). In multivariate analysis, male patients (95% confidence interval (CI): 1.945–4.249, P = 0.0001), age ≤ 60 years old (95% CI: 1.212–2.474, P = 0.003), patients with papilla stula (95% CI: 0.081–0.900, P = 0.033), and patients with a common bile duct (CBD) diameter < 12 mm (95% CI: 1.093–2.130, P = 0.013) were associated with clonorchiasis incidence. Nonclonorchiasis endoscopic diagnosis did not significantly correlate with clonorchiasis incidence (P > 0.05). Conclusions. The detection of C. sinensis eggs was significantly higher in bile than in fecal samples; thus, bile samples represent a preferable sample for the diagnosis of clonorchiasis in patients with biliary obstruction. We found that male, age ≤ 60 years old, and CBD diameter < 12 mm were independent risk factors for clonorchiasis, while papilla stula was a protective factor.

1. Introduction

Clonorchiasis, which is caused by Clonorchis sinensis, globally affects more than 15 million people, 13 million of whom live in China and other parts of East Asia [1–3]. An investigation conducted from 2005 to 2014 in Guangxi Province, southern China, showed that the prevalence of clonorchiasis in the general population reached 9.9% [4]. Clonorchiasis causes mechanical and chemical injury, resulting in inflammation [5–7], obstruction [5–8], and cancerogenesis [1, 2, 9] in the intrahepatic and extrahepatic biliary tracts [8, 10]. Common assays for diagnosing clonorchiasis include serologic detection of parasite-specific antibody and DNA [11–15], egg detection in bile and fecal samples [16, 17], and imaging [18]. The detection of C. sinensis eggs in fecal samples is specific, but with low sensitivity [19, 20], and requires skillful technique [21], especially when the infection is mild or there is biliary obstruction. Endoscopic retrograde cholangiopancreatography (ERCP) is not only a method for angiography but also a safe and effective tool for the treatment of biliary and pancreatic disorders. C. sinensis eggs are detectable in bile [6, 7, 19], but few studies have compared the detection of C. sinensis between bile and fecal samples.

It is important to evaluate independent risk factors for clonorchiasis in a large cohort of patients. Although several general risk factors for clonorchiasis have been reported [22, 23], no study to date has examined the risk factors associated with endoscopic findings. Various biliary or pancreatic disorders require different endoscopic interventions. ERCP procedures are generally safe and effective, but endoscopists must have a thorough understanding of indications for the
selected procedures. However, there are no published data that compare endoscopic manipulation with outcomes between patients with and without clonorchiasis.

This retrospective two-center study analyzed the diagnostic sensitivity by detecting *C. sinensis* eggs in bile and fecal samples and the risk factors associated with endoscopic procedures. Furthermore, endoscopic manipulation and outcomes were compared between patients with and without clonorchiasis.

2. Patients and Methods

2.1. Patients. Patients who had biliary or pancreatic disorders (including jaundice caused by biliary obstruction; clinical and biochemical or imaging data suggestive of biliary stones, tumors, and sclerosing cholangitis; pancreatic diseases including tumors, chronic pancreatitis, and pancreatic abscess; and pancreatitis of unknown etiology and sphincter of Oddi manometry) and received ERCP procedures between January 2012 and October 2018 at both the First and Second Affiliated Hospitals, Guangxi Medical University (Nanning, China), were included. Indications for ERCP followed the guidelines of the American Society of Gastrointestinal Endoscopy [24]. Patients were excluded if age < 18 years old, no detection of *C. sinensis* in both fecal and bile samples, prior ERCP, or loss of clinical data. This study protocol was approved by the Institutional Review Boards of both hospitals. Written informed consent was obtained from all participants.

2.2. Procedures. All ERCP procedures were conducted by well-trained and experienced endoscopists, who are certified to perform procedures of ERCP difficulty Grade 3 per the ERCP core curriculum [25]. The ERCP equipment involved a therapeutic duodenoscope (TJF-260V; Olympus Optical, Tokyo, Japan). Selective cannulation of the common bile duct (CBD) was performed by using a guidewire or standard catheter if patients had a preexisting sphincterotomy. All duodenoscopes were disinfected and decontaminated per the guidelines and confirmed by regular smear tests. Once guidewire cannulation was successfully established after duodenoscope entry, bile was aspirated by inserting a disposable 5F standard sphincterotome catheter into the bile duct before injection of a contrast agent for the ERCP procedure. Approximately 2–8 mL of bile (average 4 mL) was collected from patients with a clinical diagnosis of cholangitis, as suggested by clinical manifestations (jaundice, fever, and right upper quadrant pain) or radiological manifestations of biliary obstruction. The aspirated bile was immediately transferred into a sterile tube. After the injection of contrast agent, the length of the widest part of the CBD was documented and the diameter, number, and position of any CBD stone were recorded. The endoscopist on site selected endoscopic procedures including cannulation, endoscopic sphincterotomy (EST), bile culture, endoscopic papillary balloon dilation (EPBD), bougie dilatation, basket, lithotripsy basket, balloon, brush, biopsy, stent implantation, and endoscopic nasobiliary drainage (ENBD) based on the patient’s conditions and the Chinese guidelines for ERCP (2010). In this study, we used the formalin-ether concentration technique (FEC) to detect *C. sinensis* eggs in bile and/or feces for the pathogen diagnosis of clonorchiasis. The first detection of eggs in fecal samples was made before ERCP, and two more tests were repeated during hospitalization if the first detection was negative.

2.3. Observational Index. Biochemical and hematological markers were examined within 72 hours of admission before ERCP including leukocyte (white blood cell (WBC))/amylase (AMS)/total bilirubin (TBil)/direct bilirubin (DBil)/alanine aminotransferase (ALT)/aspartate transaminase (AST)/gamma glutamyl transpeptidase (GGT)/alkaline phosphatase (ALP)/carcinoembryonic antigen (CEA)/carbohydrate antigen 199 (CA-199). Demographics and clinical findings during hospitalization were collected including gender, age, endoscopic diagnosis, papilla types, CBD diameter, and CBD stone characteristics (shape, size, position, number, and color). The collection procedure and outcome data included the cannulation method, EST/EPBD/bougie dilatation/ENBD/stent implantation/brush/biopsy/bile culture, the cut size of the EST, basket/ballon/lithotripsy basket, bile culture results, brush results, immediate complications, post-ERCP pancreatitis (PEP), post-ERCP cholangitis (PEC), and serological baseline data. PEP was defined as having new or worsened abdominal pain for more than 24 h after persistent ERCP, accompanied by elevated serum amylase level more than three times the upper limit of normal. PEC was defined as having a fever > 38°C and lasting >24 h due to biliary causes after ERCP.

2.4. Statistical Analysis. Continuous variables were expressed as the mean and standard deviation or median and interquartile range, and differences were computed using the Student’s *t*-test or nonparametric test. Categorical variables were analyzed by the Pearson’s chi-square test or Fisher’s exact test. The test level in univariate was unrestricted to 0.10 if the factors underscored the clinical importance. Multivariate regression analyses were used to identify independent risk factors. Logistic regression models were employed to calculate odds ratios with 95% confidence intervals (CIs). A two-tailed *P* value < 0.05 was considered statistically significant (SPSS 22.0 for Windows, SPSS, Chicago, IL, USA).

3. Results

3.1. Baseline Characteristics. A total of 2171 consecutive patients who underwent ERCP in two hospitals between January 2012 and October 2018 were initially screened. Patients were excluded if age < 18 years old (n = 33), no detection of *C. sinensis* eggs in both bile and fecal samples (n = 822), prior ERCP (n = 161), or no clinical data (n = 36). Finally, 1119 patients were included and analyzed (Figure 1); 36.6% were female with a mean age of 57.2 ± 14.2 years (range: 20–92 years). Clonorchiasis was diagnosed in 19.2% of the 1119 patients as a result of detected *C. sinensis* eggs in the fecal and/or bile samples (Table 1).

3.2. Comparison of *C. sinensis* Egg Positivity in Bile and Feces. Among the 215 patients diagnosed with clonorchiasis, both
bile and feces were collected from 92 patients for the detection of *C. sinensis* eggs. The eggs were detected in 85/92 (85.9%) bile samples, which was significantly higher than the 58.7% (54/92) detected in feces samples (*P* = 0.001), suggesting that the sensitivity of detecting eggs in bile was significantly higher than that in feces (Tables 1 and 2).

### 4. Risk Factors for Clonorchiasis

#### 4.1. Univariate Analysis

Univariate analysis showed that gender, age (≤ 60 and > 60), endoscopic diagnosis, papilla type, CBD diameter (< 12 mm and ≥ 12 mm), and CBD stone shape were associated with clonorchiasis (*P* < 0.05, Table 3).

#### 4.2. Multivariate Analysis

Multivariate logistic regression analysis showed that gender, age, endoscopic diagnosis, papilla type, and CBD diameter were independent risk factors for clonorchiasis. The clonorchiasis prevalence in male was 2.875 times higher than that in females (95% confidence interval (CI): 1.945–4.249, *P* = 0.0001). The clonorchiasis incidence in patients ≤ 60 years old was 1.732 times higher than that in patients > 60 years old (95% CI: 1.212–2.474, *P* = 0.003). Patients with papillary fistula were less susceptible to clonorchiasis compared to those with normal papilla (95% CI: 0.081–0.900, *P* = 0.033). However, minor papilla, papillary diverticulum, and papillary carcinoma did not correlate with clonorchiasis. Patients with CBD diameter < 12 mm had a 1.526-fold higher incidence compared to those with CBD ≥ 12 mm (95% CI: 1.093–2.130, *P* = 0.013). Although endoscopic diagnosis of clonorchiasis was significantly different between the two groups (95% CI: 3.774–84.822, *P* = 0.0001), other endoscopically diagnosed diseases were not (*P* > 0.05; Table 4).

### 4.3. Comparison of Endoscopic Procedures with Clonorchiasis Incidence

Univariate analysis showed that endoscopic procedures including the cannulation method, EST, ENBD, stent implantation, and balloon and bile culture were significantly associated with clonorchiasis (*P* < 0.05). Among the 215 patients with clonorchiasis, 202 received guidewire cannulation, 11 received dual guidewire, 1 received precut papillotomy, and 1 had failed cannulation. EST and ENBD were performed for removing stones, *C. sinensis* detection, or keeping bile drainage in 178 cases. Stent was implanted in 29 cases for biliary stenosis or for drainage (28 with single plastic stent and 1 with metal stent). Ballooning was used in 164 cases for dilating the bile duct. Bile was obtained in 150 cases for culture. Other procedures showed no correlation with clonorchiasis including bougie dilatation, brush, biopsy, the cut size of EST, basket, lithotripsy basket, bile culture results, brush results, immediate complications, PEP, and PEC (*P* > 0.05; Tables 5 and 6).

### 4.4. Analysis Characteristics of the CBD Stone

CBD stones in 562 cholelithiasis patients were nonsludge. Univariate analysis showed that size, location, number, and color of these stones were not significantly associated with clonorchiasis incidence (*P* > 0.05; Table 7).

### 5. Comparison of Biochemical and Hematological Findings between Patients with and without Clonorchiasis

The analyses showed that patients in both groups had elevated liver enzymes and jaundice. WBC, DBil, and ALT levels before ERCP in patients diagnosed with clonorchiasis were significantly higher than those in nonclonorchiasis patients (*P* = 0.001, 0.022, and 0.032, respectively). AMS, TBil, AST, ALP, GGT, CEA, and CA-199 levels showed no significant correlation with clonorchiasis (*P* > 0.05; Table 8).
6. Discussion

Clonorchiasis mainly occurs in East Asia and is associated with eating raw freshwater fish that carry the parasite [22]. Guangxi Province, where our patients resided, is part of southern China, and residents enjoy raw freshwater fish. In this study, clonorchiasis was diagnosed in 19.2% of the 1119 patients as a result of detected C. sinensis eggs in the fecal and/or bile samples. In addition, clonorchiasis mainly occurred in patients younger than 60 years old (155/215, 72.1%), with a 2.875-fold higher incidence in male than female patients. Clonorchiasis incidence in this cohort was significantly higher than that reported in the general Chinese population. Fang et al. [26] reported that clonorchiasis prevalence was 2.94% and 1.84% in males and females, respectively, with the highest prevalence found in the 50- to 59-year-old age group. Hoang et al. [27] reported that the prevalence in the male was 2.33 times higher than that in females in Vietnam, which supports our finding that more male clonorchiasis patients identified in southern China were susceptible to biliary or pancreatic disorders, including jaundice and elevated hepatic biochemical markers of TBil, DBil, ALT, AST, ALP, and GGT.

Detected C. sinensis eggs is direct evidence of clonorchiasis. The egg detection methods include Kato-Katz method (KK) and direct smear microscopy (DM) [18, 28]. The sensitivities of KK, FEC, and DM reported by Manuel et al. [29] were 71%, 50%, and 3%, respectively. The so-called “gold” standard combines the following four methods: KK, spontaneous sedimentation, FEC, and DM. However, Men et al. [19] referred to a combination of six KK plus two FECT methods as the “gold” standard. However, those standards involve multiple detection methods and a collection of consecutive fecal sample detection methods, which make them difficult to apply. Furthermore, when the infection activity is low or biliary obstruction is present, the probability of detecting eggs in fecal samples is extremely low. In our study, FEC was used for the detection of C. sinensis eggs in fecal and bile samples. We found that C. sinensis eggs were significantly more frequently detected in bile (79/92, 85.9%) than in fecal (54/92, 58.7%) samples, indicating that bile detection of C. sinensis eggs is preferable to fecal detection for the

| Table 3: Univariate analysis of risk factors for clonorchiasis. | Clonorchiasis | Nonclonorchiasis | $\chi^2$ | P value |
|----------------|---------------|-----------------|---------|---------|
| Characteristic | N = 1119      |                 |         |         |
| Gender         |               |                 | 42.93   | 0.0001  |
| Male           | 178           | 532             |         |         |
| Female         | 37            | 372             |         |         |
| Age in years   |               |                 | 23.58   | 0.0001  |
| ≤60            | 155           | 487             |         |         |
| >60            | 60            | 417             |         |         |
| Endoscopic diagnosis |       |                 | 56.26   | 0.0001  |
| Choledolithiasis | 152        | 624             |         |         |
| Malignant strictures | 19        | 153             |         |         |
| Benign strictures | 10         | 44              |         |         |
| Clonorchiasis  | 15            | 3               |         |         |
| Bile duct expansions for unknown reasons | 7 | 35 |         |         |
| Pancreatic disorders | 2 | 11 |         |         |
| Normal choangiiopancreatograph | 6 | 24 |         |         |
| Else           | 4             | 10              |         |         |
| Papilla types  |               |                 | 21.10   | 0.0001  |
| Normal         | 178           | 619             |         |         |
| Minor papilla  | 5             | 40              |         |         |
| Papillary carcinoma | 2 | 43 |         |         |
| Papillary fistula | 3          | 47              |         |         |
| Papillary diverticulum | 27 | 155 |         |         |
| CBD diameter   |               |                 | 24.13   | 0.0001  |
| <12 mm         | 118           | 331             |         |         |
| ≥12 mm         | 97            | 573             |         |         |
| CBD stone shape|               |                 | 10.60   | 0.001   |
| Stone          | 94            | 468             |         |         |
| Sludge         | 58            | 156             |         |         |
diagnosis of clonorchiasis in patients with biliary obstruction. Thus, bile detection should be incorporated into the established gold standards for diagnosing clonorchiasis.

Previous studies have identified male, eating raw fish, lower educational levels, and location of the villages as demographic or epidemiologic risk factors for clonorchiasis [22, 23, 30, 31]. Multivariate analysis showed that endoscopic diagnosis of clonorchiasis was significantly different between two groups, but it was not an independent risk factor for clonorchiasis. In addition, it could be influenced by subjective judgment (such as foams and other parasites), resulting in a low sensitivity for direct diagnosis (15/41, 36.6%). Our research suggested both demographic factors of male and age ≤ 60 years old and CBD diameter < 12 mm as independent risk factors for clonorchiasis, while papilla fistula emerged as protective factor in the analysis. These findings are expected to identify a group of patients at high risk for clonorchiasis if they develop biliary or pancreatic disorders, especially in endemic regions.

When infected raw or undercooked fish is ingested by humans, the metacercariae excyst in the duodenum migrates into the intrahepatic bile ducts where eggs are laid. We wondered whether the excyst migration efficiency is related to papilla type. Our results showed that patients with normal papilla more frequently had clonorchiasis than patients with papillary fistula. As recently reported, positron emission tomography-computed tomography [32] can view the migration route within the host. This device may help investigate this issue among subjects with or without normal papilla.

We also compared the endoscopic procedures and outcomes between patients with and without clonorchiasis and found significant differences in conducting procedures including the cannulation method, EST, ENBD, stent implantation, balloon, and bile culture between the two groups. Patients with clonorchiasis were inclined to require guidewire cannulation, EST, ENBD, a single plastic stent, balloon, and bile culture. It is established that infection with C. sinensis is one of the most important factors for cholangiocarcinoma [33, 34], but our study found no significant differences in the brush results between clonorchiasis and nonclonorchiasis. The reason could be that patients with biliary or pancreatic disorders may not be representative; thus, future studies are needed to investigate the relationship between clonorchiasis and cholangiocarcinoma in a population without biliary or pancreatic disorders. There was no correlation between clonorchiasis and bile duct stones. As noted in this study, patients with clonorchiasis did not show special endoscopic features, so endoscopists must choose procedures after fully evaluating patients.

| Factors                                      | Wald    | P value   | OR       | 95% CI     |
|----------------------------------------------|---------|-----------|----------|------------|
| Gender                                       |         |           |          |            |
| Male                                         | 28.080  | 0.0001    | 2.875    | 1.945-4.249|
| Female                                       |         |           | 1        |            |
| Age in years                                 |         |           |          |            |
| ≤60                                          | 9.095   | 0.003     | 1.732    | 1.212-2.474|
| >60                                          |         |           | 1        |            |
| Endoscopic diagnosis                         |         |           |          |            |
| Cholelithiasis                               | 0.899   | 0.343     | 1.582    | 0.613-4.081|
| Malignant strictures                         | 0.108   | 0.742     | 0.838    | 0.291-2.409|
| Benign strictures                            | 0.007   | 0.932     | 1.051    | 0.329-3.358|
| Clonorchiasis                                | 13.196  | 0.0001    | 17.892   | 3.774-84.822|
| Idiopathic bile duct expansions              | 0.191   | 0.662     | 1.321    | 0.379-4.606|
| Pancreatic disorders                         | 0.088   | 0.767     | 0.763    | 0.127-4.588|
| Else                                         | 1.463   | 0.226     | 2.555    | 0.559-11.679|
| Normal cholangiopancreatography              |         |           | 1        |            |
| Papilla types                                 |         |           |          |            |
| Minor papilla                                | 2.121   | 0.145     | 0.486    | 0.184-1.283|
| Papillary carcinoma                          | 1.742   | 0.187     | 0.362    | 0.080-1.637|
| Papillary fistula                            | 4.544   | 0.033     | 0.270    | 0.081-0.900|
| Papillary diverticulum                       | 2.099   | 0.147     | 0.703    | 0.436-1.133|
| Normal papilla                               |         |           | 1        |            |
| CBD diameter                                  |         |           |          |            |
| <12 mm                                       | 6.147   | 0.013     | 1.526    | 1.093-2.130|
| ≥12 mm                                       |         |           | 1        |            |

| Table 4: Multivariate analysis of risk factors for clonorchiasis. |
A few limitations of this study are worth mentioning. First, this was a retrospective study; thus, it may inevitably produce bias in data collection and selection. Prospective, large-cohort, multicenter studies are needed to confirm our findings. Second, only one assay (i.e., FEC) was used for the detection of C. sinensis in our study, and additional

| Characteristic             | Clonorchiasis | Nonclonorchiasis | $\chi^2$ | $P$ value |
|----------------------------|---------------|------------------|----------|-----------|
| Cannulation method         |               |                  | 10.47    | 0.011     |
| Guidewire                  | 202           | 850              |          |           |
| Dual guidewire             | 11            | 24               |          |           |
| Precut papillotomy         | 1             | 29               |          |           |
| Fail                       | 1             | 1                |          |           |
| EST                        |               |                  | 13.55    | 0.0001    |
| Yes                        | 178           | 636              |          |           |
| No                         | 37            | 268              |          |           |
| Cut size of EST            |               |                  | 0.36     | 0.835     |
| Big                        | 4             | 11               |          |           |
| Medium                     | 20            | 65               |          |           |
| Small                      | 154           | 558              |          |           |
| EPBD                       |               |                  | 0.09     | 0.759     |
| Yes                        | 110           | 452              |          |           |
| No                         | 105           | 452              |          |           |
| Bougie dilatation          |               |                  | 1.93     | 0.165     |
| Yes                        | 10            | 66               |          |           |
| No                         | 205           | 838              |          |           |
| ENBD                       |               |                  | 23.42    | 0.0001    |
| Yes                        | 178           | 595              |          |           |
| No                         | 37            | 309              |          |           |
| Stent implantation         |               |                  | 20.25    | 0.0001    |
| Single plastic stent       | 28            | 189              |          |           |
| Metal stent                | 1             | 10               |          |           |
| Multiple stent             | 0             | 42               |          |           |
| No                         | 186           | 663              |          |           |
| Basket                     |               |                  | 0.67     | 0.414     |
| Yes                        | 77            | 351              |          |           |
| No                         | 138           | 553              |          |           |
| Balloon                    |               |                  | 17.45    | 0.0001    |
| Yes                        | 164           | 552              |          |           |
| No                         | 51            | 352              |          |           |
| Lithotripsy basket         |               |                  | 2.19     | 0.139     |
| Yes                        | 11            | 73               |          |           |
| No                         | 204           | 831              |          |           |
| Bile culture               |               |                  | 7.71     | 0.005     |
| Yes                        | 150           | 538              |          |           |
| No                         | 65            | 366              |          |           |
| Brush                      |               |                  | 0.02     | 0.903     |
| Yes                        | 22            | 90               |          |           |
| No                         | 193           | 814              |          |           |
| Biopsy                     |               |                  | 2.44     | 0.119     |
| Yes                        | 1             | 22               |          |           |
| No                         | 214           | 882              |          |           |
methods like KK were not used. Third, as bile samples were obtained during the ERCP procedure, the presence of *C. sinensis* eggs near the duodenal papilla may cause a positive bile finding, and ERCP procedures may have also increased the positive fecal finding. Fourth, the cohort we studied represented patients with biliary or pancreatic disorders, so these findings cannot be directly inferred to the general population.

### Table 6: Comparison of endoscopic procedure outcomes between clonorchiasis and nonclonorchiasis.

| Characteristic                | N = 1119 | Clonorchiasis | Nonclonorchiasis | $\chi^2$ | P value |
|------------------------------|----------|---------------|------------------|----------|---------|
| Bile culture results         |          |               |                  |          |         |
| Positive                     | 39       | 181           |                  |          |         |
| Negative                     | 110      | 358           |                  |          |         |
| Brush results                |          |               |                  | 0.234    | 0.628   |
| Malignant                    | 3        | 16            |                  |          |         |
| Benign                       | 19       | 73            |                  |          |         |
| Immediate complications      |          |               |                  | 1.40     | 0.237   |
| Yes                          | 2        | 23            |                  |          |         |
| No                           | 213      | 881           |                  |          |         |
| PEP                          |          |               |                  |          |         |
| Yes                          | 11       | 54            |                  |          |         |
| No                           | 204      | 850           |                  |          |         |
| PEC                          |          |               |                  | 0.0001   | 1.000   |
| Yes                          | 1        | 4             |                  |          |         |
| No                           | 214      | 900           |                  |          |         |

### Table 7: Analysis of characteristics of CBD stones between clonorchiasis and nonclonorchiasis.

| Characteristic                | N = 562 | Clonorchiasis | Nonclonorchiasis | $\chi^2$ | P value |
|------------------------------|----------|---------------|------------------|----------|---------|
| Stone size                   |          |               |                  |          |         |
| ≤5 mm                        | 15       | 52            |                  |          |         |
| ≤15 mm                       | 69       | 339           |                  |          |         |
| >15 mm                       | 10       | 77            |                  |          |         |
| Stone position in CBD        |          |               |                  | 9.76     | 0.135   |
| Upper                        | 17       | 80            |                  |          |         |
| Intermediate                 | 17       | 61            |                  |          |         |
| Lower                        | 35       | 159           |                  |          |         |
| Upper & intermediate         | 13       | 44            |                  |          |         |
| Lower & intermediate         | 7        | 75            |                  |          |         |
| Upper & lower                | 1        | 19            |                  |          |         |
| Dispersion                   | 4        | 30            |                  |          |         |
| Stone number                 |          |               |                  | 0.39     | 0.532   |
| <3                           | 65       | 308           |                  |          |         |
| ≥3                           | 29       | 160           |                  |          |         |
| Stone color                  |          |               |                  | 5.38     | 0.129   |
| Yellow                       | 16       | 57            |                  |          |         |
| Black                        | 5        | 24            |                  |          |         |
| Brown                        | 45       | 230           |                  |          |         |
| White                        | 2        | 1             |                  |          |         |

7. Conclusions

In summary, our data showed that the detection of *C. sinensis* eggs was significantly higher in bile than in fecal samples; thus, the detection of *C. sinensis* in bile represents a preferable sample for the diagnosis of clonorchiasis in patients with biliary obstruction. We also found that male, age ≤ 60 years old, and CBD diameter < 12 mm were independent risk factors.
factors for clonorchiasis while papilla fistula as protective factor, indicating that patients with normal biliary or pancreatic tract are likely to be at high risk for clonorchiasis, in addition to age and gender, especially in endemic region.

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| ERCP         | Endoscopic retrograde cholangiopancreatography |
| CBD          | Common bile duct |
| EST          | Endoscopic sphincterotomy |
| EPBD         | Endoscopic papillary balloon dilation |
| ENBD         | Endoscopic nasobiliary drainage |
| WBC          | White blood cell |
| AMS          | Amylase |
| TBil         | Total bilirubin |
| DBil         | Direct bilirubin |
| ALT          | Alanine aminotransferase |
| AST          | Aspartate transaminase |
| GGT          | Gamma glutamyl transpeptidase |
| ALP          | Alkaline phosphatase |
| CEA          | Carcinoembryonic antigen |
| CA-199       | Carbohydrate antigen 199 |
| PEP          | Post-ERCP pancreatitis |
| PEC          | Post-ERCP cholangitis |
| FEC          | Formalin-ether concentration technique |
| KK           | Kato-Katz method |
| DM           | Direct smear microscopy |

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Ethical Approval**

This study was approved by the Ethics Committee of the Second Affiliated Hospital and the First Affiliated Hospital of Guangxi Medical University, Nanning, China.

**Conflicts of Interest**

The authors declare no conflict of interest.

**Authors’ Contributions**

Qin MB and Huang JA conceived the study. Liao GL conducted the study and wrote the manuscript. Liao GL, Ruan HQ, Peng P, Liu SQ, Qin JF, Liang ZH, Tang GD, and Qin MB collected and analyzed the data. All authors contributed to the design and interpretation of the results and manuscript preparation. Liao GL and Ruan HQ contributed equally to this study. Qin MB and Huang JA were the guarantors and corresponding authors.

**References**

[1] T. Furst, J. Keiser, and J. Utzinger, “Global burden of human food-borne trematodiasis: a systematic review and meta-analysis,” *The Lancet Infectious Diseases*, vol. 12, no. 3, pp. 210–221, 2012.

[2] M. B. Qian, Y. D. Chen, S. Liang, G. J. Yang, and X. N. Zhou, “The global epidemiology of clonorchiasis and its relation with cholangiocarcinoma,” *Infectious Diseases of Poverty*, vol. 1, no. 1, p. 4, 2012.

[3] M. B. Qian, Y. D. Chen, and F. Yan, “Time to tackle clonorchiasis in China,” *Infectious Diseases of Poverty*, vol. 2, no. 1, 2013.

[4] D. H. Lai, X. K. Hong, B. X. Su et al., “Current status of Clonorchis sinensis and clonorchiasis in China,” *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 110, no. 1, pp. 21–27, 2016.

[5] D. Choi, J. H. Lim, K. T. Lee et al., “Gallstones and Clonorchis sinensis infection: a hospital-based case-control study in Korea,” *Journal of Gastroenterology and Hepatology*, vol. 23, Part 2, pp. e399–e404, 2008.

[6] T. Qiao, R. H. Ma, X. B. Luo, Z. L. Luo, and P. M. Zheng, “Cholecystolithiasis is associated with Clonorchis sinensis infection,” *PloS One*, vol. 7, no. 8, p. e42471, 2012.

[7] T. Qiao, R. H. Ma, Z. L. Luo, L. Q. Yang, X. B. Luo, and P. M. Zheng, “Clonorchis sinensis eggs are associated with calcium carbonate gallbladder stones,” *Acta Tropica*, vol. 138, pp. 28–37, 2014.

[8] B. I. Choi, J. K. Han, S. T. Hong, and K. H. Lee, “Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis,” *Clinical Microbiology Reviews*, vol. 17, no. 3, pp. 540–552, 2004.

**Table 8: Comparison of biochemical and hematologic findings between clonorchiasis and nonclonorchiasis.**

| Characteristics | Clonorchiasis | Nonclonorchiasis | P value |
|----------------|--------------|------------------|---------|
| WBC (10^9/L)   | 8.8 (6.6–11.2) | 7.6 (5.6–9.9)   | 0.001   |
| AMS            | 90.0 (52.0–193.3) | 74.0 (51.0–123.0) | 0.090   |
| TBil (µmol/L)  | 96.9 (26.6–171.9) | 58.0 (20.1–160.2) | 0.054   |
| DBil (µmol/L)  | 68.9 (16.4–126.9) | 39.4 (10.1–117.3) | 0.022   |
| ALT (U/L)      | 87.0 (34.3–190.0) | 67.0 (30.0–134.0) | 0.032   |
| AST (U/L)      | 65.0 (33.5–109.5) | 53.0 (30.0–100.0) | 0.086   |
| ALP (U/L)      | 189.5 (120.3–284.5) | 193.0 (118.0–334.0) | 0.464   |
| GGT (U/L)      | 310.5 (162.8–570.5) | 297.0 (128.0–576.0) | 0.890   |
| CEA (ng/mL)    | 2.3 (1.4–3.0) | 2.0 (1.2–3.6) | 0.542   |
| CA-199 (U/mL)  | 32.9 (10.3–139.1) | 28.3 (9.0–133.4) | 0.526   |

All data are represented by median and interquartile range (IQR).
[9] H. R. Shin, J. K. Oh, M. K. Lim et al., “Descriptive epidemiology of cholangiocarcinoma and clonorchiasis in Korea,” Journal of Korean Medical Science, vol. 25, no. 7, pp. 1011–1016, 2010.

[10] B. Sripan, S. Kaewkes, P. M. Intapan, W. Maleewong, and P. J. Brindley, “Food-borne trematodiasis in Southeast Asia epidemiology, pathology, clinical manifestation and control,” Advances in Parasitology, vol. 72, pp. 305–350, 2010.

[11] M. H. Choi, I. C. Park, S. Li, and S. T. Hong, “Excretory-secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA,” The Korean Journal of Parasitology, vol. 41, no. 1, pp. 35–39, 2003.

[12] Y. J. Kim, S. M. Lee, G. E. Choi et al., “Performance of an enzyme-linked immunosorbent assay for detection of Clonorchis sinensis infection in high- and low-risk groups,” Journal of Clinical Microbiology, vol. 48, no. 7, pp. 2365–2367, 2010.

[13] T. Y. Kim, P. Y. Cho, J. W. Na, and S. J. Hong, “Molecular cloning and phylogenetic analysis of Clonorchis sinensis elongation factor-1α,” Parasitology Research, vol. 101, no. 6, pp. 1557–1562, 2007.

[14] C. Shen, J. A. Lee, S. R. Allam et al., “Serodiagnostic applicability of recombinant antigens of Clonorchis sinensis expressed by wheat germ cell-free protein synthesis system,” Diagnostic Microbiology and Infectious Diseases, vol. 64, no. 3, pp. 334–339, 2009.

[15] J. W. Ju, H. N. Joo, M. R. Lee et al., “Identification of a serodiagnostic antigen, legumain, by immunoproteomic analysis of excretory-secretory products of Clonorchis sinensis adult worms,” Proteomics, vol. 9, no. 11, pp. 3066–3078, 2009.

[16] R. J. Traub, J. Macaranas, M. Munghin et al., “A new PCR-based approach indicates the range of Clonorchis sinensis now extends to Central Thailand,” PLoS Neglected Tropical Diseases, vol. 3, no. 1, p. e367, 2009.

[17] T. H. Le, N. Van De, D. Blair, P. Sithithaworn, and D. P. McManus, “Clonorchis sinensis and Opisthorchis viverrini: Development of a mitochondrial-based multiplex PCR for their identification and discrimination,” Experimental Parasitology, vol. 112, no. 2, pp. 109–114, 2006.

[18] J. H. Kim, M. H. Choi, Y. M. Bae, J. K. Oh, M. K. Lim, and S. T. Hong, “Correlation between discharged worms and fecal egg counts in human clonorchiasis,” PLoS Neglected Tropical Diseases, vol. 5, no. 10, e1339, 2011.

[19] K. R. Joo and S. J. Bang, “A bile based study of Clonorchis sinensis infections in patients with biliary tract diseases in Ulsan, Korea,” Yonsei Medical Journal, vol. 46, no. 6, pp. 794–798, 2005.

[20] M. B. Qian, P. Yap, Y. C. Yang et al., “Accuracy of the Kato-Katz method and formalin-ether concentration technique for the diagnosis of Clonorchis sinensis, and implication for assessing drug efficacy,” Parasites & Vectors, vol. 6, no. 1, p. 314, 2013.

[21] M. V. Johansen, P. Sithithaworn, R. Bergquist, and J. Utzinger, “Towards improved diagnosis of zoonotic trematode infections in Southeast Asia,” Advances in Parasitology, vol. 73, pp. 171–195, 2010.

[22] M.-B. Qian, J. Utzinger, J. Keiser, and X.-N. Zhou, “Clonorchiasis,” The Lancet, vol. 387, no. 10020, pp. 800–810, 2016.

[23] V. T. Phan, A. K. Ersboll, D. T. Do, and A. Dalsgaard, “Raw-fish-eating behavior and fishborne zoonotic trematode infec-

tion in people of northern Vietnam,” Foodborne Pathogens and Disease, vol. 8, no. 2, pp. 255–260, 2011.

[24] T. H. Baron, B. T. Petersen, K. Mergener et al., “Quality indicators for endoscopic retrograde cholangiopancreatography,” Gastrointestinal Endoscopy, vol. 63, 4 Suppl, pp. S29–S34, 2006.

[25] ASGE Training Committee, J. Jorgensen, N. Kubiliun et al., “Endoscopic retrograde cholangiopancreatography (ERCP): core curriculum,” Gastrointestinal Endoscopy, vol. 83, no. 2, pp. 279–289, 2016.

[26] Y. Y. Fang, Y. D. Chen, X. M. Li, J. Wu, Q. M. Zhang, and C. W. Ruan, “Current prevalence of Clonorchis sinensis infection in endemic areas of China,” Chinese Journal of Parasitology & Parasitic Diseases, vol. 26, no. 2, pp. 99–103, 109, 2008.

[27] H. Q. Vinh, W. Phimphraphi, S. Tangkawattana et al., “Risk factors for Clonorchis sinensis infection transmission in humans in northern Vietnam: a descriptive and social network analysis study,” Parasitology International, vol. 66, no. 2, pp. 74–82, 2017.

[28] S. T. Hong, M. H. Choi, C. H. Kim, B. S. Chung, and Z. Ji, “The Kato-Katz method is reliable for diagnosis of Clonorchis sinensis infection,” Diagnostic Microbiology and Infectious Disease, vol. 47, no. 1, pp. 345–347, 2003.

[29] M. Calvopina, D. Romero-Alvarez, F. Diaz, W. Cevallos, and H. Sugiyama, “A comparison of Kato-Katz technique to three other methods for diagnosis of Amphimerus spp, liver fluke infection and the prevalence of infection in Chachi Amerindians of Ecuador,” PLoS One, vol. 13, no. 10, article e0203811, 2018.

[30] M. B. Qian, Y. D. Chen, Y. Y. Fang et al., “Disability weight of Clonorchis sinensis infection: captured from community study and model simulation,” PLoS Neglected Tropical Diseases, vol. 5, no. 12, p. e1377, 2011.

[31] M. B. Qian, Y. D. Chen, Y. Y. Fang et al., “Epidemiological profile of Clonorchis sinensis infection in one community, Guangdong, People’s Republic of China,” Parasites & Vectors, vol. 6, no. 1, p. 194, 2013.

[32] T. I. Kim, W. G. Yoo, B. K. Kwak, J.–W. Seok, and S.–J. Hong, “Tracing of the bile-chemotactic migration of juvenile Clonorchis sinensis in rabbits by PET-CT,” PLoS Neglected Tropical Diseases, vol. 5, no. 12, p. e1414, 2011.

[33] C. de Martel, M. Plummer, and S. Franceschi, “Cholangiocarcinoma: descriptive epidemiology and risk factors,” Gastroentérologie Clinique et Biologique, vol. 34, no. 3, pp. 173–180, 2010.

[34] P. Prueksapanich, P. Piyachaturawat, P. Aumpansub, W. Ridtitid, R. Chaiteerakij, and R. Rerknimitr, “Liver fluke-associated biliary tract cancer,” Gut Liver, vol. 12, no. 3, pp. 236–245, 2018.