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Association between eosinophil count and cholelithiasis among a population with *Clonorchis sinensis* infection in Foshan City, China

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Abstract

The association between eosinophil count and cholelithiasis among people with *Clonorchis sinensis* infection is still uncertain. We conducted a cross-sectional study to investigate the associations among *Clonorchis sinensis* infection, eosinophil count and cholelithiasis. The study included 4628 participants from January to December 2018. The levels of eosinophil count were divided into four groups according to the quartiles of eosinophil count. Spearman's rank correlation was performed to assess the association between eosinophil counts and *Clonorchis sinensis* egg counts. Multiple regression analysis was performed to evaluate the relationships among *C. sinensis* infection, eosinophil count and cholelithiasis after adjusting for three models. The prevalence of *C. sinensis* infection was 38.72% (1792/4628), and the prevalence of cholelithiasis was 6.03% (279/4628). The infection rate of *C. sinensis* was higher in the cholelithiasis group than in the non-cholelithiasis group (63.08% vs. 37.16%, $P < 0.001$). Significant differences were found among various eosinophil count quartiles for *C. sinensis* infection, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γ-GT), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (CRE), blood urea nitrogen (BUN), uric acid (UA) and non-alcoholic fatty liver disease (NAFLD) (all $P < 0.001$). A significant positive correlation was found between eosinophil count and log-transformed *C. sinensis* egg count ($r = 0.9477$, $P < 0.001$). Multiple logistic regression analysis revealed that light and moderate intensities of *C. sinensis* infection were associated with cholelithiasis ($P < 0.01$ and $P < 0.001$, respectively), and *C. sinensis* infection with eosinophil count ranging from 0.05 to 0.5×10^9/l were associated with cholelithiasis ($P < 0.05$). In conclusion, our findings suggest that the light and moderate infections of *C. sinensis* with eosinophil count ranging from 0.05 to 0.5×10^9/l may be associated with a higher risk of cholelithiasis.

Clonorchiasis is mainly prevalent in Asian countries and regions, including South Korea, China, northern Vietnam and far-eastern Russia (Lun *et al.*, 2005; Furst *et al.*, 2012; Qian *et al.*, 2016). China has the largest population of infected people, which is estimated at 13 million (Qian *et al.*, 2012, 2016). The high infection rate of clonorchiasis is mainly attributed to the habit of consuming raw or inadequately cooked freshwater fish from ponds and lakes contaminated with sewage (Lo *et al.*, 2013). Patients with *Clonorchis sinensis* infection are often asymptomatic or have unspecific symptoms, which may easily lead to missed diagnosis or misdiagnosis (Lun *et al.*, 2005). Chronic clonorchiasis caused by *C. sinensis* infection results in various complications in the liver and biliary systems, mainly including cholelithiasis, cholangitis, cholecystitis and biliary tract obstruction (Lai *et al.*, 2007). Stool examination by the Kato–Katz (KK) method is non-invasive and inexpensive for the convenient detection of *C. sinensis* eggs (Hong & Fang, 2012; Tang *et al.*, 2016). It has been demonstrated that the KK method is sensitive and reliable for diagnosing clonorchiasis (Hong *et al.*, 2003).

Cholelithiasis is one of the most frequent complications of *C. sinensis* infection (Tang *et al.*, 2016). Cholelithiasis includes intrahepatic stones (IHSs), common bile duct (CBD) stones and gallstones. Cholelithiasis has become a major health problem because it can lead to cholecystitis, cholangitis, pancreatitis and even cholangiocarcinoma (Lai *et al.*, 2007). The presence of gallstones in clonorchiasis has been documented and the patients with calcium carbonate gallbladder stones have been reported to have higher infection rate of *C. sinensis* (Ma *et al.*, 2015). Furthermore, accumulating studies have shown that cholelithiasis is closely associated with chronic *C. sinensis* infection (Choi *et al.*, 2008; Chen *et al.*, 2019).

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The relationship between chronic C. sinensis infection and cholelithiasis has been reported in several studies (Choi *et al.*, 2008, 2012; Qiao *et al.*, 2014; Chen *et al.*, 2019), though other investigations have found no significant relationship between cholelithiasis and clonorchiasis (Hou *et al.*, 1989; Kim *et al.*, 2009). In most of these prior studies, there were insufficient samples of patients infected with *C. sinensis*. Therefore, the relationship between *C. sinensis* infection and cholelithiasis is not yet well understood. The eosinophil count (EOS) is a stable, readily available and inexpensive marker of parasitic infection, which is also an important predictor of asthma, drug hypersensitivity reactions, neoplasm, connective-tissue disorders, primary hypereosinophilic syndromes and transplant rejection (Hogan *et al.*, 2008; Liao *et al.*, 2016). To our knowledge, there is no literature on the association between eosinophil count and cholelithiasis among people with *C. sinensis* infection. Therefore, we conducted a single-centre cross-sectional study in Chinese population to investigate the associations among cholelithiasis, eosinophil count and *C. sinensis* infection.

Material and methods

Study population

A total of 4987 participants were recruited from the Health Examination Centre of Guangdong Integrated Hospital of Traditional Chinese and Western Medicine, Foshan City, Guangdong Province, China, from January 2018 to December 2018. All participants underwent stool examination for *C. sinensis* infection, ultrasonography for non-alcoholic fatty liver disease (NAFLD) and cholelithiasis, as well as blood test for eosinophil count and metabolic parameters. Trained medical staff used a questionnaire to collect participants' information and medical history, including age, gender, smoking status, alcohol intake, history of gallstone surgery, history of chronic kidney disease, history of hepatobiliary surgery, history of cancer and history of viral hepatitis. Participants with any of the following characteristics were excluded from the study: (1) alcohol consumption of 2 or more drink units per week; (2) history of chronic kidney disease; (3) history of hepatobiliary surgery; (4) history of cancer; (5) history of viral hepatitis. The study was reviewed and approved by the ethics committee of Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine. All participants signed informed consent before the examinations.

Data collection

The blood pressure of participants was measured after at least 15 min of seated rest. Body weight and standing height were measured without shoes and outerwear. Body mass index (BMI) was defined as weight divided by height squared (kg/m^2). Venous blood samples were collected after an overnight fast of 8 h. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γ-GT), triglyceride (TG), total cholesterol (TC), fasting plasma glucose (FPG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (CRE), blood urea nitrogen (BUN) and uric acid (UA) levels were measured using an Olympus AU-640 autoanalyser (Olympus, Japan). Eosinophil count was measured using Sysmex 2100 whole blood cell analyser (Sysmex, Japan). NAFLD and cholelithiasis were determined by abdominal ultrasonography with ACUSON X150 ultrasound system (Siemens, Japan). Stool examination was performed with the KK method for diagnosis of *C. sinensis* infection in each participant. For stool examination, triplicate Kato-Katz thick smears were prepared using standard 41.7 mg templates, and the number of *C. sinensis* eggs was counted and recorded under a microscope by experienced technicians (Qian *et al.*, 2013). The intensity of *C. sinensis* infection was expressed by eggs per gram of faeces (EPG) and classified into three categories according to World Health Organization (World, 2012): light (1–1999 EPG), moderate (2000–3999 EPG) and heavy (≥4000 EPG). All the test results were obtained from the laboratory of Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine.

Statistical analysis

Normal distribution data were described as mean ± standard deviation (SD), and the t-test was used to compare the groups. Median (interquartile range) was used to describe the data with skewed distribution, and the groups were compared with Wilcoxon's rank-sum test. Qualitative data are expressed in frequency (percentage) and compared with the chi-square test. In addition, the data were further divided into four groups according to the quartiles of EOS: G1, EOS# $< 0.05 \times 10^9$/l; G2, 0.05×10^9/l ≤ EOS# $< 0.1 \times 10^9$/l; G3, 0.1×10^9/l ≤ EOS# $< 0.5 \times 10^9$/l; G4, EOS# $\geq 0.5 \times 10^9$/l. The Kruskal–Wallis test was used to compare different quartiles of eosinophil count. Spearman's rank correlation was performed to assess the association between eosinophil counts and *C. sinensis* egg counts. In addition, we used forward stepwise multiple logistic regression models to estimate the associations between *C. sinensis* infection, eosinophil count and cholelithiasis. The association between the intensity of *C. sinensis* infection and cholelithiasis was analysed using a multiple logistic regression model. Multivariable models were adjusted as follows: Model 1 was adjusted for age and gender; Model 2 was adjusted for Model 1 + BMI, smoking status, systolic blood pressure and diastolic blood pressure; Model 3 was adjusted for Model 2 + ALT, AST, ALP, γ-GT, TC, TG, FPG, HDL-C, LDL-C, CRE, BUN, UA and NAFLD. All data analyses were conducted using SPSS Statistics 22.0 (IBM, USA). A two-tailed test was used with a significance level of 0.05.

Results

Clinical and demographic characteristics of the study participants

Overall, 4628 Chinese participants (3221 males and 1407 females) were enrolled (fig. 1). The clinical and demographic data are shown in table 1. *Clonorchis sinensis* infection was present in 38.72% (1792/4628) of the participants. Cholelithiasis was present in 6.03% (279/4628) of the participants, 63.08% (176/279) of whom were infected with *C. sinensis*. The average ages of the cholelithiasis group and non-cholelithiasis group were 43.2 ± 6.4 years and 34.5 ± 5.3 years, respectively ($P < 0.001$). The infection rate of *C. sinensis* in cholelithiasis group was significantly higher than that in non-cholelithiasis group (63.08% vs. 37.16%, $P < 0.001$). Moreover, the prevalence of cholelithiasis was higher in participants with light, moderate and heavy intensities of *C. sinensis* infection compared with non-cholelithiasis group (12.19% vs. 8.99%, 36.20% vs. 18.44%, and 14.70% vs. 9.73%, respectively), although the difference was not significant. In addition, eosinophil count in participants with cholelithiasis was significantly higher than that in

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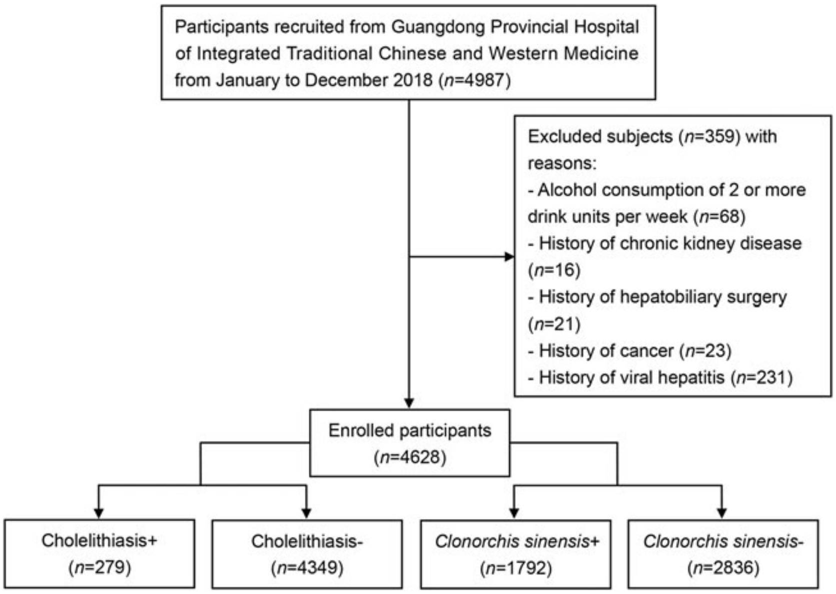

Fig. 1. Flow chart of participant selection.

those without cholelithiasis ($P < 0.001$). There were no differences between participants with and without cholelithiasis in terms of gender, ALT, AST, HDL-C, LDL-C, CRE and UA (all $P > 0.05$).

Characteristics of the study participants according to eosinophil count quartile

The data were subdivided into four groups according to the quartiles of eosinophil count (table 2). There were significant differences among various eosinophil count quartiles for gender, age, smoking status, BMI, DBP, SBP, ALT, AST, ALP, γ-GT, TC, TG, HDL, LDL, CRE, BUN, UA and NAFLD (all $P < 0.001$). Moreover, there was a significant difference among various eosinophil count quartiles for *C. sinensis* infection ($P < 0.001$).

Association between eosinophil count and Clonorchis sinensis infection

To further investigate the association between *C. sinensis* infection and eosinophil count levels, participants from the *C. sinensis* positive group and negative group were subdivided into four grades according to eosinophil count quartiles, and the percentages of each grade in the two groups are shown in fig. 2. In the *C. sinensis* negative group, the percentages of G1, G2, G3 and G4 were 4.89%, 20.51%, 70.14% and 4.47%, respectively. In the *C. sinensis* positive group, the percentages of G1, G2, G3 and G4 were 3.64%, 9.36%, 77.35% and 9.64%, respectively. There was a significant difference between the two groups for the percentages of eosinophil count quartiles ($\chi^2 = 141.1$, $P < 0.001$). As shown in fig. 3a, the differences among the eosinophil counts of light, moderate and heavy intensities of *C. sinensis* infection were significant ($P < 0.001$). In addition, a significant positive correlation was found between log-transformed *C. sinensis* egg counts and eosinophil counts ($r = 0.9477$, $P < 0.001$, fig. 3b).

Association between the intensity of Clonorchiasis sinensis *infection and cholelithiasis*

We established a multiple logistic regression to evaluate the association between the intensity of *C. sinensis* infection and cholelithiasis. As shown in table 3, the light and moderate intensities of *C. sinensis* infection were significantly associated with higher risk of cholelithiasis (β = 1.90, 95% CI = 1.26–2.85, $P = 0.002$; β = 3.13, 95%CI = 2.34–4.18, $P < 0.001$) in Model 1. And further adjustment for BMI, smoking status, SBP and DBP (Model 2), and additional adjustment for other clinical variables (Model 3), the light and moderate intensities of *C. sinensis* infection were still significantly associated with cholelithiasis. However, there was no association between cholelithiasis and the heavy intensity of *C. sinensis* infection.

Association between C. sinensis *infection, eosinophil count and cholelithiasis*

We further established a multiple logistic regression to evaluate the association between *C. sinensis* infection, eosinophil count and cholelithiasis. As shown in table 4, multiple logistic regression analysis revealed that there was no association between eosinophil count and cholelithiasis in the participants without *C. sinensis* infection. But *C. sinensis* infection with eosinophil count at G2 level ($0.05–0.1 \times 10^9$/l) was significantly associated with cholelithiasis after adjusting for pertinent clinical variables, and the β values of Model 1, Model 2 and Model 3 were 2.52, 2.48 and 2.49 (95% CI: 0.87–7.14; 0.87–7.05; 0.87–7.12, all $P < 0.05$), respectively. *C. sinensis* infection with eosinophil count at the G3 level ($0.1–0.5 \times 10^9$/l) was significantly associated with cholelithiasis after adjusting for pertinent clinical variables, and the β values of Model 1, Model 2 and Model 3 were 2.687, 2.63 and 2.50 (95% CI: 1.07–6.76; 1.04–6.61; 0.99–6.35, all $P < 0.05$), respectively. However, there was no association between cholelithiasis and *C. sinensis* infection with eosinophil count at G1 or G4 levels ($<0.05 \times 10^9$/l, $\geq 0.5 \times 10^9$/l, respectively).

Discussion

Clonorchis sinensis is mainly prevalent in Asian countries and regions, including South Korea, China, northern Vietnam and far-eastern Russia, and 200 million people worldwide are at risk of *C. sinensis* infection (Hong & Fang, 2012; Qian *et al.*, 2016). It has been reported that more than 15 million people are infected

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Table 1. Characteristics of the study participants with and without cholelithiasis.

Parameters	Cholelithiasis+ ($n = 279$)	Cholelithiasis- ($n = 4349$)	P value
Gender, male, *n* (%)	198 (70.97)	3023 (69.51)	0.608
Age, years	43.2 ± 6.4	34.5 ± 5.3	<0.001
Smoking, *n* (%)	66 (23.66)	752 (17.29)	<0.001
BMI, kg/m^2	24.3 (18.59–35.3)	23.8 (12–46.3)	<0.001
SBP, mmHg	122 (114–132)	119 (111–127)	<0.001
DBP, mmHg	75 (70–81)	73 (68–79)	<0.001
ALT, U/L	21 (14–29)	22 (15–29)	0.667
AST, U/L	20 (12–24)	21 (17–24)	0.548
ALP, U/L	91.1 (84–99)	81.4 (88.2–95.4)	<0.001
γ-GT, U/L	29 (21–42)	26 (19–39)	<0.001
EOS# (×10^9/l)	0.2 (0.12–0.31)	0.18 (0.11–0.28)	<0.001
TG, mmol/l	1.57 (1.12–2.53)	1.39 (0.94–2.15)	<0.001
TC, mmol/l	5.43 (4.76–6.16)	5.25 (4.64–5.95)	<0.001
FPG, mg/dl	5.26 (4.89–5.61)	5.04 (4.75–5.42)	<0.001
HDL-C, mmol/l	1.41 (1.22–1.68)	1.43 (1.23–1.67)	0.303
LDL-C, mmol/l	3.22 (2.67–3.76)	3.12 (1.83–3.34)	0.091
CRE, μmol/l	77.7 (63.9–92.1)	78.6 (65.3–89.4)	0.707
BUN, mmol/l	4.66 (3.94–5.49)	4.53 (3.83–5.29)	0.047
UA, μmol/l	391.1 (331.0–467.8)	389.2 (326.6–460.3)	0.406
NAFLD, *n* (%)	117 (41.94)	1287 (29.59)	<0.001
C. sinensis+, *n* (%)	176 (63.08)	1616 (37.16)	<0.001
Intensity of *C. sinensis* infection			0.136
Light (1–1999 EPG)	34 (12.19%)	391 (8.99%)	
Moderate (2000–3999 EPG)	101 (36.20%)	802 (18.44%)	
Heavy (≥4000 EPG)	41 (14.70%)	423 (9.73%)	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GT, γ-glutamyltranspeptidase; EOS#, eosinophil count; TG, triglyceride; TC, total cholesterol; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid; NAFLD, non-alcoholic fatty liver disease; *C. sinensis*, *Clonorchis sinensis*; EPG, eggs per gram of faeces.

with *C. sinensis*, of which 1.5 million to 2 million have clinical symptoms or complications (Hong & Fang, 2012). Among them, approximately 13 million people in China were infected with *C. sinensis* (Furst *et al*., 2012). In China, Guangdong Province is the most infected province, followed by Guangxi, Heilongjiang and Jilin Province (Chen *et al*., 2012). Foshan, as the third largest city in the Pearl River Delta of Guangdong Province, is a severe epidemic area of *C. sinensis* (Yuan *et al*., 2018).

In our study, we found that patients with cholelithiasis were generally older than non-cholelithiasis patients (43.2 ± 6.4 years vs. 34.5 ± 5.3 years, $P < 0.001$). Moreover, cholelithiasis was more prevalent in patients who smoked (23.66% vs. 17.29%, $P < 0.01$) and had higher BMI (24.3 kg/m^2 vs. 23.8 kg/m^2, $P < 0.001$). In addition, compared with non-cholelithiasis patients, cholelithiasis patients had higher SBP (122 mmHg vs. 119 mmHg, $P < 0.001$) and DBP (75 mmHg vs. 73 mmHg, $P < 0.001$). These findings reflect that cholelithiasis may be closely associated with metabolic disorders or cardiovascular diseases (Zamani *et al*., 2014).

Studies on the relationship between *C. sinensis* infection and cholelithiasis are inconsistent and controversial. Some studies have shown that no significant relationship between cholelithiasis and clonorchiasis (Hou *et al*., 1989; Kim *et al*., 2009). But other studies have shown that *C. sinensis* infection is closely related to cholelithiasis (Choi *et al*., 2008; Tang *et al*., 2016). *Ascaris lumbricoides* and *C. sinensis* have been occasionally identified in the biliary tracts or the gallbladder in Asians, and it has been reported that *C. sinensis* can provide the dead bodies or the eggs as the nidus for stone formation in the bile ducts or the gallbladder (Qiao *et al*., 2009; Ma *et al*., 2015). It is believed that adult *C. sinensis* can migrate to biliary tree, including gallbladder, intrahepatic and extrahepatic bile ducts, after being ingested in duodenum. In addition, it has been proposed that *C. sinensis* can cause severe adenomatous hyperplasia of the biliary epithelium, mucin hypersecretion, inflammation and fibrosis around the duct, and deposit body or eggs in the biliary stent, thus contributing to the development of cholelithiasis (Hong & Fang, 2012). Therefore, it is necessary to further explore the association between the *C. sinensis* infection and cholelithiasis.

In our study, the results showed that the infection rate of *C. sinensis* in patients with cholelithiasis was significantly higher than that in patients without cholelithiasis (63.08% vs. 37.16%,

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Table 2. Characteristics of the study participants according to eosinophil count quartile.

	EOS# quartiles (×10^9/l)				
Parameters	G1 (<0.05)	G2 (0.05–0.1)	G3 (0.1–0.5)	G4 (≥0.5)	*P* value
Number	170	750	3409	299	NA
Gender, male, *n* (%)	76 (44.7)	408 (54.4)	2467 (72.4)	270 (90.3)	<0.001
Age, years	38.4 ± 5.2	40 ± 6.2	43 ± 4.8	45 ± 5.1	<0.001
Smoking, *n* (%)	12 (7.1)	97 (12.9)	653 (19.08)	56 (18.5)	<0.001
BMI, kg/m^2	21.8 (20.10–23.91)	22.89 (20.94–24.65)	23.98 (22.06–25.59)	24.59 (22.88–26.25)	<0.001
SBP, mmHg	116 (109.61–127.82)	116.40 (109.0–125.0)	119.52 (111.27–128.0)	121.24 (113.34–130.97)	<0.001
DBP, mmHg	71.0 (66.88–76.91)	71.39 (66.0–77.0)	73.60 (68.0–79.0)	75 (70.0–81.46)	<0.001
ALT, U/l	16 (12–22)	18 (13–26)	20 (15–30)	22 (16–33)	<0.001
AST, U/l	19 (17–22)	20 (15–30)	20 (14–24)	21 (18–25)	<0.001
ALP, U/l	84.3 (79.4–91)	85.8 (80.3–92)	88.7. (81.9–96)	95.0 (87.0–104.2)	<0.001
γ-GT, U/l	20 (15.0–30.0)	23.0 (16–33)	27.0 (19.0–41.0)	32.5 (23.0–49.0)	<0.001
TC, mmol/l	5.16 (4.42–5.81)	5.12 (4.53–5.77)	5.29 (4.58–6.0)	5.36 (4.62–6.09)	<0.001
TG, mmol/l	1.11 (0.76–1.61)	1.15 (0.8–1.7)	0.99 (1.46–2.28)	1.62 (1.12–2.43)	<0.001
FPG, mg/dl	5.09 (4.81–5.42)	5.06 (4.78–5.42)	5.05 (4.75–5.43)	5.02 (4.70–5.45)	0.434
HDL, mmol/l	1.62 (1.40–1.84)	1.54 (1.31–1.73)	1.41 (1.22–1.64)	1.35 (1.14–1.58)	<0.001
LDL, mmol/l	2.99 (2.49–3.59)	3.06 (2.57–3.60)	3.21 (2.71–3.76)	3.32 (2.72–3.97)	<0.001
CRE, μmol/l	67.4 (55.4–83.0)	72.7 (59.7–85.55)	79.4 (66.3–90.1)	83.75 (74.8–92.5)	<0.001
BUN, mmol/l	4.27 (3.53–5.05)	4.40 (3.67–5.14)	4.57 (3.89–5.34)	4.56 (3.97–5.32)	<0.001
UA, μmol/l	329.6 (279.0–412.8)	356.95 (295.35–432.4)	394.35 (335.1–464.8)	421.6 (356.8–489.2)	<0.001
NAFLD, *n* (%)	32 (18.8)	167 (22.3)	1090 (32.0)	115 (38.5)	<0.001
C. sinensis+, *n* (%)	30 (17.6)	167 (22.3)	1421 (41.6)	174 (58.2)	<0.001

EOS#, eosinophil count; NA, not applicable; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GT, γ-glutamyltranspeptidase; TG, triglyceride; TC, total cholesterol; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid; NAFLD, non-alcoholic fatty liver disease; *C. sinensis*, *Clonorchis sinensis*.

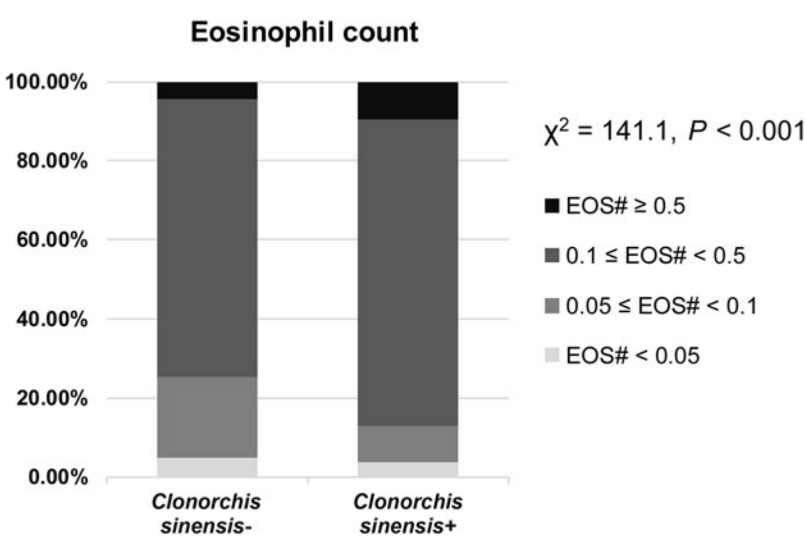

Fig. 2. The percentages of eosinophil count quartiles in *Clonorchis sinensis* positive and negative group. The data were divided into four groups according to the quartiles of eosinophil count. In the *C. sinensis* negative group, the percentages of G1, G2, G3 and G4 were 4.89%, 20.51%, 70.14% and 4.47%, respectively. In the *C. sinensis* positive group, the percentages of G1, G2, G3 and G4 were 3.64%, 9.36%, 77.35% and 9.64%, respectively.

$P < 0.001$). In addition, multiple logistic regression analysis indicated that the light and moderate intensities of *C. sinensis* infection were significantly associated with cholelithiasis ($P < 0.01$ and $P < 0.001$, respectively). Therefore, our data suggest that *C. sinensis* infection, especially light and moderate intensities of the infection, is associated with cholelithiasis. This finding may be related to the characteristics of liver fluke infection. It has been proposed that early infection of liver fluke has an initially

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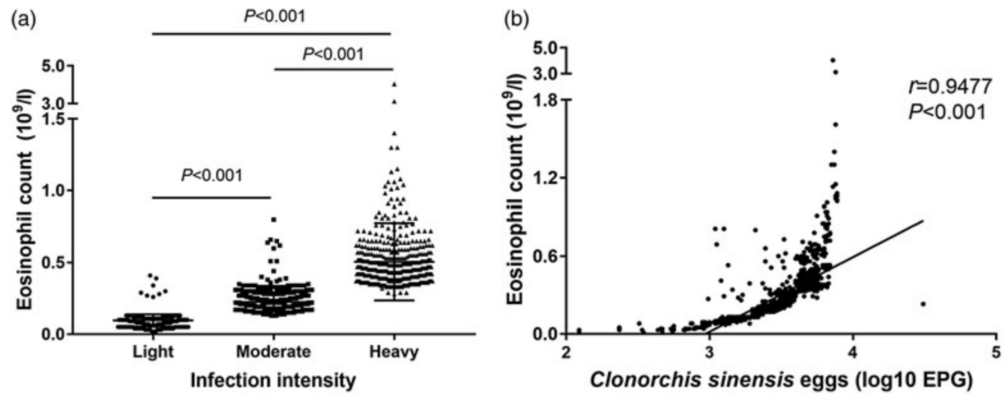

Fig. 3. The association between eosinophil count and *Clonorchis sinensis* infection. (a) Comparison of eosinophil counts from participants with different intensities of *C. sinensis* infection. (b) Correlation between *C. sinensis* egg counts and eosinophil counts. *r* = Spearman's rank correlation coefficient, EPG = eggs per gram of faeces.

Table 3. Multiple logistic regression analysis of different intensity of *Clonorchiasis sinensis* infection as risk factors of cholelithiasis.

	Model 1		Model 2		Model 3	
Variables	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
No infection	reference	NA	reference	NA	reference	NA
Light (1–1999 EPG)	1.90 (1.26–2.85)	0.002	1.89 (1.26–2.83)	0.002	1.84 (1.22–2.27)	0.004
Moderate (2000–3999 EPG)	3.13 (2.34–4.18)	<0.001	3.08 (2.31–4.12)	<0.001	3.03 (2.26–4.07)	<0.001
Heavy (≥4000 EPG)	1.56 (1.02–2.40)	0.41	1.52 (0.99–2.33)	0.57	1.46 (0.94–2.25)	0.114

Model 1 was adjusted for age and gender.
Model 2 was adjusted for Model 1 + body mass index, smoking status, systolic blood pressure, diastolic blood pressure.
Model 3 was adjusted for Model 2 + alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, triglyceride, total cholesterol, fasting blood glucose, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, blood urea nitrogen, uric acid, non-alcoholic fatty liver disease.
Abbreviations: β, odds ratio; CI, confidence interval; NA, not applicable; EPG, eggs per gram of faeces.

Table 4. Multiple logistic regression analysis of a combination of *Clonorchiasis sinensis* infection and eosinophil count as risk factors of cholelithiasis.

	Model 1		Model 2		Model 3	
Variables	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Clonorchis sinensis – and EOS#($<0.05 \times 10^9$/l)	reference	NA	reference	NA	reference	NA
Clonorchis sinensis – and EOS#($0.05–0.1 \times 10^9$/l)	1.27 (0.47–3.42)	0.635	1.28 (0.44–3.32)	0.640	1.25 (0.46–3.37)	0.663
Clonorchis sinensis – and EOS#($0.1–0.5 \times 10^9$/l)	1.05 (0.41–2.67)	0.919	1.04 (0.41–2.65)	0.932	1.02 (0.40–2.61)	0.964
Clonorchis sinensis – and EOS#($\geq 0.5 \times 10^9$/l)	1.13 (0.32–4.08)	0.849	1.12 (0.31–4.02)	0.866	1.05 (0.29–3.80)	0.946
Clonorchis sinensis + and EOS#($<0.05 \times 10^9$/l)	2.01 (0.37–11.05)	0.422	1.98 (0.36–10.88)	0.433	1.42 (0.23–8.92)	0.711
Clonorchis sinensis + and EOS#($0.05–0.1 \times 10^9$/l)	2.52 (0.87–7.14)	0.022	2.48 (0.87–7.05)	0.032	2.49 (0.87–7.12)	0.041
Clonorchis sinensis + and EOS#($0.1–0.5 \times 10^9$/l)	2.69 (1.07–6.76)	0.026	2.63 (1.04–6.61)	0.030	2.50 (0.99–6.35)	0.034
Clonorchis sinensis + and EOS#($\geq 0.5 \times 10^9$/l)	1.74 (0.59–5.17)	0.09	1.68 (0.57–5.00)	0.122	1.48 (0.49–4.47)	0.131

Model 1 was adjusted for age and gender.
Model 2 was adjusted for Model 1 + body mass index, smoking status, systolic blood pressure, diastolic blood pressure.
Model 3 was adjusted for Model 2 + alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, triglyceride, total cholesterol, fasting blood glucose, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, blood urea nitrogen, uric acid, non-alcoholic fatty liver disease.
Abbreviations: EOS#, eosinophil count; β, odds ratio; CI, confidence interval; NA, not applicable.

higher rate of egg production, which will reduce over time (Walker *et al.*, 2006; Valero *et al.*, 2011). Therefore, it is possible that patients with more *C. sinensis* eggs in faeces may be at an early stage of infection and have not yet developed cholelithiasis.

It has been widely recognized that *C. sinensis* and other parasitic infections can lead to elevated eosinophils (O'Connell & Nutman, 2015; Kovalszki & Weller, 2016). Eosinophils exert a range of biological effects against helminth parasites, leading to

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inflammation and damage of affected tissues. Eosinophilia in helminth infections is typically associated with a pronounced T helper 2 (Th2) immune response including the production of interleukin-4 (IL-4), interleukin-5(IL-5) and interleukin-13 (IL-13), as well as immunoglobulin E and the expansion and mobilisation of other specific effector cells, such as mast cells and basophils (Maizels *et al.*, 2004). Until recently, eosinophil has been mainly considered as the end-stage effector cell with cytotoxic activities consequent upon release of the toxic cationic proteins stored in their secondary granules. These non-specific killing abilities are thought to have evolved as a host defence mechanism against large non-phagocytosable parasites (Ueki *et al.*, 2016). Our results also indicated that the participants infected with *C. sinensis* had higher percentages of G3 and G4 levels of eosinophil count when compared with normal participants. In addition, Spearman's rank correlation analysis showed that *C. sinensis* egg count was positively associated with eosinophil count ($r = 0.9477$, $P < 0.001$). However, there are few studies concerning the associations among *C. sinensis*, eosinophil count and cholelithiasis.

The pathological study of gallbladder infected by *C. sinensis* showed that eosinophils and lymphocytes could infiltrate the gallbladder wall, but the proliferation of epithelial cells was not obvious. *Clonorchis sinensis* multiplies in a large number of intrahepatic bile ducts, blocking the bile duct lumen, resulting in obstructed bile excretion and abnormal bacterial reproduction. *Clonorchis sinensis* eggs, dead worms, exfoliated biliary epithelia, bacteria and other synergistic action promote the formation of gallstones (Qiao *et al.*, 2012; Urdaneta & Casadesus, 2017). Eosinophils are multipotent white blood cells with many functions, such as initiating inflammation and regulating immune response. Hypereosinophilia is a common phenomenon which has a protective effect in the process of parasitic infection. It can lead to inflammation and damage of affected biliary tree and promote the development of cholelithiasis (Chen *et al.*, 2019). This also indicates that *C. sinensis* infection may be closely related to eosinophil count and cholelithiasis.

In our study, the KK method was used to examine *C. sinensis* eggs for the diagnosis of *C. sinensis* infection, and ultrasonography was used for the diagnosis of cholelithiasis. Our results suggest that *Cl. sinensis* infection and eosinophil count are associated with cholelithiasis after adjusting the pertinent clinical variables, including age, gender, BMI, smoking status, SBP, DBP, ALT, AST, ALP, γ-GT, TC, TG, FPG, HDL-C, LDL-C, CRE, BUN, UA and NAFLD. However, there was no association between eosinophil count and cholelithiasis when *C. sinensis* infection was negative. Similarly, when *C. sinensis* infection was positive and eosinophil count was less than 0.05×10^9/l or more than 0.5×10^9/l, there was no association between eosinophil count and cholelithiasis. Interestingly, *C. sinensis* infection with eosinophil count in $0.05–0.1 \times 10^9$/l or $0.1–0.5 \times 10^9$/l level was significantly associated with cholelithiasis. This result suggest that the clonorchiasis patients with eosinophil count ranging from $0.05–0.5 \times 10^9$/l may be at higher risk of cholelithiasis.

We have to note that there are some limitations in our research. (1) Subjects were recruited from the physical education centre of our hospital. Most of the participants were examined by enterprises and institutions as a group. This part of the population has a high level of economic income, which may not represent the general population. (2) In this study, the KK method was used to detect faecal eggs under a microscope. There was a certain rate of missed detection and false negatives of *C. sinensis*, especially when the number of *C. sinensis* eggs was very low (Hong *et al.*, 2003). (3) This is a cross-sectional study which could only investigate the relationship between *C. sinensis* infection and cholelithiasis.

In conclusion, our data show that the participants infected with *C. sinensis* are more likely to develop cholelithiasis. The light and moderate intensities of *C. sinensis* infection with eosinophil count ranging from 0.05 to 0.5×10^9/l may be associated with a higher risk of cholelithiasis, suggesting that early detection of eosinophil count may be useful for the assessment of potential cholelithiasis risk in patients infected with *C. sinensis*. But future studies are needed to clarify the causal relationship between *C. sinensis* infection and cholelithiasis. In addition, biochemical studies are needed to better understand the pathophysiology behind the role of *C. sinensis* infection in cholelithiasis.

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Conflicts of interest. None.

Ethical standards. This study was approved by the Ethics Committee of Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine. All participants were informed regarding voluntary participation and they were advised that they could withdraw from the study at any time. All data were kept confidential.

References

Chen YD, Zhou CH and Xu LQ (2012) Analysis of the results of two nationwide surveys on Clonorchis sinensis infection in China. *Biomedical and Environmental Sciences* **25**, 163–166.

Chen CH, Lin CL, Hsu CY and Kao CH (2019) A retrospective administrative database analysis of the association between clonorchiasis or helminthiasis and the development of cholelithiasis. *Current Medical Research and Opinion* **35**, 489–496.

Choi D, Lim JH, Lee KT, *et al.* (2008) Gallstones and Clonorchis sinensis infection: A hospital-based case-control study in Korea. *Journal of Gastroenterology and Hepatology* **23**, e399–e404.

Furst T, Keiser J and Utzinger J (2012) Global burden of human food-borne trematodiasis: A systematic review and meta-analysis. *Lancet Infectious Diseases* **12**, 210–221.

Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, Kay AB and Rothenberg ME (2008) Eosinophils: Biological properties and role in health and disease. *Clinical and Experimental Allergy* **38**, 709–750.

Hong ST and Fang Y (2012) Clonorchis sinensis and clonorchiasis, an update. *Parasitology International* **61**, 17–24.

Hong ST, Choi MH, Kim CH, Chung BS and Ji Z (2003) The Kato-Katz method is reliable for diagnosis of Clonorchis sinensis infection. *Diagnostic Microbiology and Infectious Disease* **47**, 345–347.

Hou MF, Ker CG, Sheen PC and Chen ER (1989) The ultrasound survey of gallstone diseases of patients infected with Clonorchis sinensis in southern Taiwan. *The Journal of Tropical Medicine and Hygiene* **92**, 108–111.

Kim HG, Han J, Kim MH, *et al.* (2009) Prevalence of clonorchiasis in patients with gastrointestinal disease: A Korean nationwide multicenter survey. *World Journal of Gastroenterology* **15**, 86–94.

Kovalszki A and Weller PF (2016) Eosinophilia. *Prim Care* **43**, 607–617.

Lai CH, Chin C, Chung HC, Liu H, Hwang JC and Lin HH (2007) Clonorchiasis-associated perforated eosinophilic cholecystitis. *American Journal of Tropical Medicine and Hygiene* **76**, 396–398.

Liao W, Long H, Chang CC and Lu Q (2016) The eosinophil in health and disease: From bench to bedside and back. *Clinical Reviews in Allergy & Immunology* **50**, 125–139.

Lo TC, Chang JH, Lee HH and Kuo HW (2013) Risk factors for and prevalence of clonorchiasis in Miaoli County, Taiwan. *The Southeast Asian Journal of Tropical Medicine and Public Health* **44**, 950–958.
Lun ZR, Gasser RB, Lai DH, Li AX, Zhu XQ, Yu XB and Fang YY (2005) Clonorchiasis: A key foodborne zoonosis in China. *Lancet Infectious Diseases* **5**, 31–41.
Ma RH, Qiao T, Luo ZL, Luo XB, Zheng PM and Yang LQ (2015) Gallbladder stone types in patients with cholelithiasis and Clonorchis sinensis infection in the endemic area of clonorchiasis Chinese. *Journal of Parasitology and Parasitic Diseases* **33**, 167–171.
Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD and Allen JE (2004) Helminth parasites–masters of regulation. *Immunological Reviews* **201**, 89–116.
O'Connell EM and Nutman TB (2015) Eosinophilia in infectious diseases. *Immunology and Allergy Clinics of North America* **35**, 493–522.
Qian MB, Chen YD, Liang S, Yang GJ and Zhou XN (2012) The global epidemiology of clonorchiasis and its relation with cholangiocarcinoma. *Infectious Diseases of Poverty* **1**, 4.
Qian MB, Yap P, Yang YC, *et al.* (2013) Accuracy of the Kato-Katz method and formalin-ether concentration technique for the diagnosis of Clonorchis sinensis, and implication for assessing drug efficacy. *Parasit Vectors* **6**, 314.
Qian MB, Utzinger J, Keiser J and Zhou XN (2016) Clonorchiasis. *Lancet* **387**, 800–810.
Qiao T, Ma RH, Zhang YD, Feng YY, Wang XQ and Wang XF (2009) Clonorchis sinensis eggs involved in the formation of gallbladder stones. *China Journal of Modern Medicine* **19**, 2094–2096.
Qiao T, Ma RH, Luo XB, Luo ZL and Zheng PM (2012) Cholecystolithiasis is associated with Clonorchis sinensis infection. *PLoS One* **7**, e42471.
Qiao T, Ma RH, Luo ZL, Yang LQ, Luo XB and Zheng PM (2014) Clonorcis sinensis eggs are associated with calcium carbonate gallbladder stones. *Acta Tropica* **138**, 28–37.
Tang ZL, Huang Y and Yu XB (2016) Current status and perspectives of Clonorchis sinensis and clonorchiasis: Epidemiology, pathogenesis, omics, prevention and control. *Infectious Diseases of Poverty* **5**, 71.
Ueki S, Tokunaga T, Fujieda S, Honda K, Hirokawa M, Spencer LA and Weller PF (2016) Eosinophil ETosis and DNA traps: A new look at eosinophilic inflammation. *Current Allergy and Asthma Reports* **16**, 54.
Urdaneta V and Casadesus J (2017) Interactions between bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. *Frontiers in Medicine* **4**, 163.
Valero MA, Panova M, Perez-Crespo I, Khoubbane M and Mas-Coma S (2011) Correlation between egg-shedding and uterus development in Fasciola hepatica human and animal isolates: Applied implications. *Veterinary Parasitology* **183**, 79–86.
Walker SM, Hoey E, Fletcher H, Brennan G, Fairweather I and Trudgett A (2006) Stage-specific differences in fecundity over the life-cycle of two characterized isolates of the liver fluke, Fasciola hepatica. *Parasitology* **133**, 209–216.
World Health Organization (2012) Soil-transmitted helminthiases: eliminating as public health problem soil-transmitted helminthiases in children: progress report 2001–2010 and strategic plan 2011–2020.
Yuan R, Huang J, Zhang X and Ruan S (2018) Modeling the transmission dynamics of Clonorchiasis in Foshan, China. *Scientific Reports* **8**, 15176.
Zamani F, Sohrabi M, Alipour A, *et al.* (2014) Prevalence and risk factors of cholelithiasis in Amol city, northern Iran: A population based study. *Archives of Iranian Medicine* **17**, 750–754.