

## RESEARCH ARTICLE

# Effects of co-infection with *Clonorchis sinensis* on the sex hormones levels in male patients with chronic hepatitis B

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**Abstract**

**Objective:** We evaluated the levels of sex hormones in male hepatitis B patients co-infected with *Clonorchis sinensis* (*C. sinensis*).

**Methods:** A total of 136 male individuals were enrolled in this study, including 27 healthy controls, 28 patients with *C. sinensis* mono-infection, 19 patients with only chronic hepatitis B, 18 patients with post-hepatitis B liver cirrhosis, 26 chronic hepatitis B patients co-infected with *C. sinensis*, and 18 post-hepatitis B liver cirrhosis patients coinfecting with *C. sinensis*. Serum levels of progesterone (P), luteinizing hormone (LH), estradiol (E2), testosterone (T), prolactin (PRL), and follicle stimulating hormone (FSH) in these groups were measured.

**Results:** The results showed that compared with the LC group, the LC<sup>+</sup> *C. sinensis* co-infected group had an increase in E2 but decrease in T and FSH. The levels of E2 in CHB<sup>+</sup> *C. sinensis* co-infected patients were significantly higher than those in CHB mono-infected patients, but the significantly lower levels of T were observed. Compared with HCs group, the LC group showed significant increase in all terms of sex hormones, except PRL. By contrast, the CHB mono-infected group presented an apparent decrease in E2, T, and PRL than the HCs group. However, there were no significant differences in sex hormone levels between the *C. sinensis* mono-infected patients and HCs.

**Conclusion:** This study suggests that *C. sinensis* co-infection aggravates the sex hormone disturbance in HBV patients at both chronic hepatitis and cirrhosis stages, providing evidences for potential strategies in disease prevention and treatment.

**KEYWORDS**

chronic hepatitis B, *Clonorchis sinensis*, co-infection, post-hepatitis B liver cirrhosis

## 1 | INTRODUCTION

Hepatitis B virus (HBV) is a small hepatotropic DNA virus that mainly results in acute and chronic hepatitis B (CHB) in infected individuals.<sup>1</sup>

While the consciousness of environmental health has increased and HBV vaccines are widely available, the incidence of HBV infection continues to increase. As previously reported by the World Health Organization, there are over 257 million people infected with HBV

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around the world, and the count of deaths due to HBV-related liver diseases such as cirrhosis and liver cancers is about 0.88 million.<sup>2</sup> Epidemiological research studies in China have estimated 120 million people carrying the hepatitis B surface antigen (HBsAg), 20 million people with chronic hepatitis B (CHB), and 300,000 patients per year dying of HBV infection and its complications.<sup>3</sup> Guangdong Province is one of the most popular areas of HBV infection in China and has witnessed 12.38 million HBsAg carriers and 61.59 million people with HBV infection history (estimated in 2009), leading to high mortality and society burden.<sup>4</sup>

*Clonorchiasis* is a severe zoonotic parasitic disease caused by *Clonorchis sinensis* (*C. sinensis*) in the hepatobiliary duct of humans and other mammals.<sup>5</sup> It is prevalent in east Asia and southeast Asia such as China, South Korea, Vietnam, Laos, and the Russian Far East.<sup>6</sup> There have been over 200 million people globally at risk of *C. sinensis* infection, while infected patients have exceeded 15 million.<sup>7</sup> The endemic areas in China include 27 provinces, cities, and autonomous regions, with about 12.50 million people infected by *C. sinensis*, accounting for 85% of total infected sufferers worldwide, the *C. sinensis* infected number has been estimated to be 6 million.<sup>8,9</sup> Accumulative studies have found that chronic infection of *C. sinensis* induces a string of hepatobiliary diseases such as hepatic fibrosis, gallstones, and choledocholithiasis and even leads to liver cirrhosis and hepatobiliary carcinoma. Moreover, recurrent infection in endemic area populations can promote the progress of hepatic fibrosis constantly, which brings devastating disease burdens for both patients and the society.<sup>10-13</sup>

Co-infection of HBV and *C. sinensis* is not rare in endemic areas of China with male predominance.<sup>14,15</sup> These two pathogens can both lead to chronic infection developing liver dysfunction and even hepatocellular carcinoma (HCC).<sup>16</sup> Since the liver is the center of sex hormone metabolism, chronic liver diseases with different causes can affect the transformation and inactivation of sex hormones and further lead to endocrine disturbance. Previous studies have demonstrated sex hormone disturbance as a common manifestation of CHB, liver cirrhosis, HCC, and other severe liver diseases.<sup>17,18</sup> The alterations in serum levels of sex hormones have been tightly linked to the development of HBV-related diseases, the disease severity, and even the risk of HCC.<sup>19,20</sup> However, the related mechanisms involved in the associations between sex hormones and HBV infection remain unclear. Co-infection with other type of pathogens such as *C. sinensis*, *schistosomiasis* and *Plasmodium spp.* have potential effects on the disease state and treatment response in HBV patients and some others.<sup>16,21,22</sup> However, these studies mainly focus on the alterations of HBV DNA levels and transaminase. Although serum levels of sex hormones are also an important index of liver functions, to our knowledge, there is a lack of exploration in the features of sex hormones in hepatitis B patients co-infected with *C. sinensis*. In the present study, we included relevant subjects from the Third Affiliated Hospital of Sun Yat-sen University from 2019 to 2021 and analyzed their serum levels of sex hormones. In addition, our preliminary study only included male participants.

## 2 | MATERIALS AND METHOD

### 2.1 | Study participants

From January 1st, 2019 to December 31st, 2021, a total of 136 men among both outpatients and inpatients in the Third Affiliated Hospital of Sun Yat-sen University were included in our study. And, their serum samples were collected. All participants were diagnosed by experienced experts according to diagnosis criteria and then selected under inclusion and exclusion criteria. They were finally classified into six groups: post-hepatitis B liver cirrhosis patients with *C. sinensis* co-infection (LC<sup>+</sup> *C. sinensis*, *N* = 18), chronic hepatitis B (CHB) patients co-infected with *C. sinensis* (CHB<sup>+</sup> *C. sinensis*, *N* = 26), patients with single post-hepatitis B liver cirrhosis (LC, *N* = 18), patients with only CHB (CHB, *N* = 19), patients with *C. sinensis* mono-infection (*C. sinensis*, *N* = 28), and health controls (HCs, *N* = 27). The study protocol was approved by the ethics committee of The Third Affiliated Hospital of Sun Yat-sen University. All participants signed the written informed consent.

In addition, we conducted the fecal *C. sinensis* egg examination in all subjects to confirm the presence of *C. sinensis* infection. The inclusion criterion for patients who were only infected with

*C. sinensis* was *C. sinensis* eggs-positive. The inclusion criteria for patients who were only infected with HBV and post-hepatitis B liver cirrhosis were the following: HBV surface-antigen (HBsAg)-positive; and HBV DNA >20IU/ml. Post-hepatitis B liver cirrhosis was defined clinically as at least two of the following five criteria being met: (1) image studies for diagnosing LC are CT, abdominal ultrasound, and MRI. Findings of these images are nodular liver surface, splenomegaly, and the presence of increasing portal venous pressure; (2) endoscopy revealed esophagus and stomach varicose vein of the fundus; (3) liver-stiffness measurement was consistent with cirrhosis; (4) serum albumin (Alb) <35.0 g/L or prothrombin time prolonged >3 s; (5) platelet (Plt) count <100 × 10<sup>9</sup>/L or pathological diagnosis were consistent with liver cirrhosis. The inclusion criteria for patients who were co-infected were HBsAg-positive, HBV DNA >20IU/ml, and *C. sinensis* eggs-positive. All HCs had to fulfill the included criteria: negative for both HBsAg in serum and *C. sinensis* egg in stool. In addition, mono-infected patients with HBV or post-hepatitis B liver cirrhosis and co-infected patients were prescribed antiviral drugs and symptomatic treatment. Neither mono-infected with *C. sinensis* nor co-infected patients were treated with anthelmintic treatment. Exclusion criteria for all participants were as follows: (i) with history of thyroid diseases, diabetes, hypophysis, or cardiovascular diseases; (ii) diagnosed with alcoholic hepatitis or its induced cirrhosis; (iii) with hepatitis or cirrhosis caused by other virus; (iv) with malignancies in digestive system.

### 2.2 | Serum sex hormone assay

Peripheral venous blood samples were collected after the subjects fasted for one night with additive-free dried tubes. Serum samples

were extracted freshly, and quantitative analysis of serum sex hormones was conducted. Six sex hormones including progesterone (P), luteinizing hormone (LH), estradiol (E2), testosterone (T), prolactin (PRL), and follicle stimulating hormone (FSH) were detected using a chemiluminescence method on Architect-i2000 (Abbott Laboratories).

## 2.3 | Statistical analysis

Variables including age, *C. sinensis* egg count, and HBsAg level were expressed as median (range), while all sex hormone indices (not normally distributed) were presented as median  $\pm$  interquartile range (IQR). The Kruskal–Wallis rank test was used for comparison among multiple groups. Rank-based ANOVA was conducted for comparison between two groups, and the data were analyzed using GRAPHPAD PRISM 7.0. *p* values  $<.05$  were considered statistically significant.

## 3 | RESULTS

### 3.1 | General information

General information of study participants are presented in Table 1, including age in all groups and results of both fecal *C. sinensis* egg count and HBsAg level in the patient groups. Results showed that there were no significant differences in age among six groups.

### 3.2 | Sex hormone levels in different groups

As shown in Figure 1, comparisons in the levels of six sex hormones were performed between each two of these six groups. We pairwise compared the serum levels of sex hormones between single infected groups with co-infected groups. Compared with mono-infected LC patients, LC<sup>+</sup> *C. sinensis* co-infected patients showed higher E2,

accompanied with lower T and FSH, and the differences were significant ( $p = .016, .000, \text{ and } .035$ , respectively). In CHB patients co-infected with *C. sinensis*, the E2 significantly increased, while the T decreased when compared with the simple CHB group ( $p = 0.000$  and  $.006$ , respectively).

To further explore the relationships between *C. sinensis* infection and alterations of sex hormones, we also compared each mono-infected groups with HCs. The results showed that compared with HCs, the HBV mono-infected LC group showed increases in all sex hormones with statistical significance, except PRL (all  $p <.05$ ). In the CHB mono-infected group, the levels of E2, T, and PRL were lower than those in the HC group, and the differences were significant ( $p = .042, .044, \text{ and } .028$ , respectively). However, the *C. sinensis* mono-infected patients showed similar levels in all six sex hormones with HCs. All comparison results of sex hormones between two groups are summarized in Table 2.

## 4 | DISCUSSION

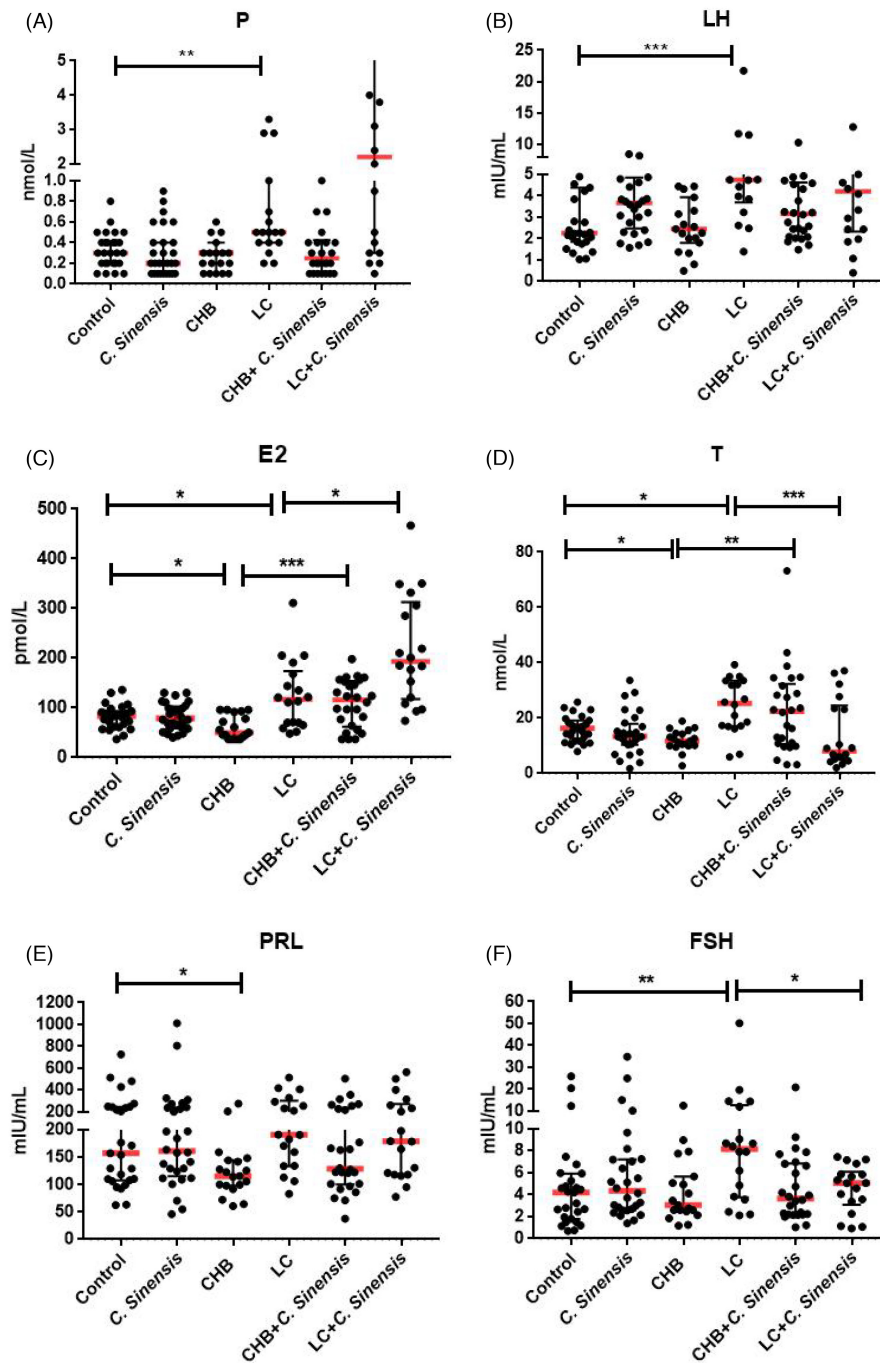
Guangdong Province have been considered to have a high prevalence of hepatitis B and *Clonorchiasis*, and co-infection of HBV and *C. sinensis* is also common.<sup>23</sup> It attaches great importance to paying attention on the susceptible population in this area. Previous studies have found that HBV damages liver function in host and further develops hepatitis, cirrhosis, and even HCC. Since the liver plays a key role in sex hormone metabolism, HBV infection can present with sex hormone disturbance in different degrees. However, it remains unclear whether the *C. sinensis* infection could also affect sex hormone levels in *C. sinensis* mono-infected patients and HBV<sup>+</sup> *C. sinensis* co-infected patients. In the present study, we preliminarily explored the relationships among *C. sinensis* infection, HBV, and sex hormones in male patients. Features of sex hormones in six different groups were illustrated. To our knowledge, it is the first study investigating the sex hormone levels in male patients with co-infection of HBV and *C. sinensis*. Our results may help clinicians understand the effects

TABLE 1 Normal information of study participants

Classification diagnosis (Group)	N	Age (year)	Fecal <i>C. sinensis</i> egg count (n/g)	HBs Ag (IU/ml)
LC <sup>+</sup> <i>C. sinensis</i> co-infection	18	52.00 (38–68)	200.00 (100–5000)	405.00 (64.40–1432.00)
CHB <sup>+</sup> <i>C. sinensis</i> co-infection	26	46.00 (33–63)	100.00 (100–300)	2405.00 (244.00–10846.00)
LC (HBV mono-infection)	18	54.50 (39–73)		204.50 (19.24–721.80)
CHB (HBV mono-infection)	19	38.00 (29–67)		176.00 (20.00–5024.00)
<i>C. sinensis</i> mono-infection	28	51.50 (19–76)	100.00 (100–5000)	
HCs	27	47.50 (27–76)		

Abbreviations: CHB: chronic hepatitis B; *C. sinensis*: *Clonorchis sinensis*; HCs: healthy controls; LC: liver cirrhosis.

Note: Data are expressed as the median (range).



**FIGURE 1** Serum sex hormones levels in the HCs group, *C. sinensis* mono-infected, CHB mono-infected group, CHB<sup>+</sup> *C. sinensis* co-infected, LC group, LC<sup>+</sup> *C. sinensis* co-infected group. (A) Serum progesterone levels in six groups. (B) Serum LH levels in six groups. (C) Serum estradiol levels in six groups. (D) Serum testosterone levels in six groups. (E) Serum pituitary prolactin levels in six groups. (F) Serum follicle stimulating hormone levels in six groups. Data show the median  $\pm$  interquartile range. Asterisks indicate statistically significant differences between Two groups, as measured by Kruskal-Wallis rank test (\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ ).

of *C. sinensis* infection on the HBV-related diseases and provide evidences for further prevention and treatment for mono- and co-infected patients.

Among these sex hormones, T is the most active androgen in humans, while E2 is the most active estrogen. In a healthy human body, 95% of serum T is synthesized and secreted by testicular stromal cells. T can transform into E2 with aromatase in peripheral tissues, which accounts for 50%~70% of total E2, while the rest is produced in adrenal cortex.<sup>24</sup> Previous studies have found that serum T levels gradually elevated in male patients with CHB, compensated cirrhosis, and decompensated cirrhosis after hepatitis B.<sup>25</sup> The present study showed that compared with HCs, all sex hormones including

LH, PRL, E2, T, FSH, and P increased in patients with only post-hepatitis B cirrhosis, while E2, T, and PRL decreased in simple CHB patients. Some researchers have also found that the HBsAg titer in the serum of male patients is positively correlated with the level of T while negatively correlated with E2. Similarly, animal studies in adult male mice showed that the levels of sex hormones are related with replication of HBV-DNA in the liver and high HBsAg level. After gonadectomy, their sex hormone levels decrease, accompanied with reduced replication levels of HBV. However, these alterations are not observed in immature male mice.<sup>26</sup> Moreover, Breidbart et al.<sup>27</sup> have observed that treatment with T has no effects on the HBsAg levels in mice with testicular feminization mutation, while in normal

TABLE 2 Comparison of sex hormones between two groups

	LH	E2	T	FSH	PRL	P
LC <sup>+</sup> <i>C. sinensis</i> vs. LC	-	↑	↓	↓	-	-
CHB <sup>+</sup> <i>C. sinensis</i> vs. CHB	-	↑	↓	-	-	-
Mono-LC vs. HCs	↑	↑	↑	↑	-	↑
Mono-CHB vs. HCs	-	↓	↓	-	↓	-

Abbreviations: CHB, chronic hepatitis B; *C. sinensis*, *Clonorchis sinensis*; E2, estradiol; FSH, follicle stimulating hormone; HCs, healthy controls; LC, liver cirrhosis; LH, luteinizing hormone; P, progesterone; PRL, pituitary prolactin; T, testosterone.

male mice, T significantly increases the produce of HBsAg. It suggests that the effects of T on the HBsAg levels is mediated by androgen receptors in the liver. Considering that the T level increased in LC patients while decreased in LC<sup>+</sup> *C. sinensis* patients, alterations of T may be a promising biomarker in co-infection. A previous study has indicated that *C. sinensis* coinfection affects the disease state and treatment response in HBV patients,<sup>16</sup> which may support our supposition. However, a larger sample size needs to be enrolled in a further study to make sure the specific relationships between them. In the present study, E2 significantly decreased in the CHB group but increased in post-hepatitis B cirrhosis patients. Combined with previous evidences, we speculated that increased activity of aromatase, portosystemic shunting, and elevated sex hormone binding globulin (SHBG) in cirrhosis patients lead to an increase in E2 by promoting the T2's transforming to E2, reducing the inactivation of E2 in the liver and decreasing E2's binding to globulin.<sup>25,28</sup>

Furthermore, serum PRL in hepatitis and cirrhosis has also been related with the severity of liver dysfunction, in line with our findings. The sensitivity of PRL cells may increase in these patients following the elevated E2 and reduced T, so their serum PRL increases correspondingly. In addition, the liver is the main organ for PRL degradation. Liver cirrhosis could affect this progress and further enhance the serum PRL level. It has been generally believed that the high estrogen level induced by its decreased inactivation in the liver leads to cirrhosis's triple signs including liver palm, spider nevus, and male breast development in male patients. The increases of P, LH, and FSH in the post-hepatitis B cirrhosis group shown in our results may further provide evidence for these feminization manifestations in male patients.

We found that there was no difference in the levels of sex hormones between the in *C. sinensis* mono-infected patients and HCs, which implies that mono-infection by *C. sinensis* does not affect sex hormones. However, cirrhosis patients co-infected with *C. sinensis* showed higher E2 with lower T and FSH than HBV mono-infected cirrhosis patients, and *C. sinensis* co-infected CHB patient also had higher E2 and lower T than patients with only CHB. It suggests that co-infection with *C. sinensis* in CHB may further expand damages in the liver, promote hepatic fibrosis, induce cirrhosis and liver cancers, and also aggravate the disturbance in sex hormones. Therefore, *C. sinensis* co-infection promotes feminization symptoms and sexual

dysfunction in male chronic HBV patients, exerting effects on therapies and prognosis of the diseases. Control and prevention of *C. sinensis* coinfection are essential in the control and prevention of HBV infection. And, evaluations of serum T and E2 may be potential biomarkers for monitoring liver damage in CHB patients coinfecting with *C. sinensis*.

## 5 | CONCLUSION

In this study, we found that sex hormone levels in mono-infected HBV patients were distinct from those in HCs, and they also showed alterations in *C. sinensis*/HBV coinfecting patients compared with mono-infected HBV patients. Our results not only highlight the importance of timely treatment and careful assessment of sex hormones for *C. sinensis* coinfecting HBV patients but also provide basis for further studies in this field.

### AUTHOR CONTRIBUTIONS

Study design: BH. Information collection: WC. Sample assessment: ML. Formal analysis: MS. Supervision: BH. Funding acquisition: HD. Writing—original draft: HD. Writing—review and editing: HD and MS.

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### CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

### DATA AVAILABILITY STATEMENT

The data in the current study are available from the corresponding author on reasonable request." cd\_value\_code="text

### THE LIMITATIONS OF THE STUDY

Our results not only highlight the importance of timely treatment and careful assessment of sex hormones for *C. sinensis* coinfecting HBV patients but also provide the basis for further studies in this field. However, as a preliminary study, it had several limitations. There are few cases studied in our paper, and there is not enough further research on the mechanism. So, explorations into underlying mechanisms for these features in coinfecting patients are awaited.

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