INTRODUCTION

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

Clonorchiasis is a severe zoonotic parasitic disease caused by *Clonorchis sinensis* (*C. sinensis*) in the hepatobiliary duct of humans and other mammals. It is prevalent in east Asia and southeast Asia such as China, South Korea, Vietnam, Laos, and the Russian Far East. There have been over 200 million people globally at risk of *C. sinensis* infection, while infected patients have exceeded 15 million. 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. Accumulative studies have found that chronic infection of *C. sinensis* induces a string of hepatobiliary diseases such as hepatic fibrosis, gallstones, and cholelithiasis 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.

Co-infection of HBV and *C. sinensis* is not rare in endemic areas of China with male predominance. These two pathogens can both lead to chronic infection developing liver dysfunction and even hepatocellular carcinoma (HCC). 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. 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. 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. 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.

## 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*+, *C. sinensis*, *N* = 18), chronic hepatitis B (CHB) patients co-infected with *C. sinensis* (*CHB* + *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 × 10⁹/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 ± 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+ C. sinensis co-infected patients showed higher E2, accompanied with lower T and FSH, and the differences were significant (\( p = .016, .000, \) 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 (\( p = .042, .044, \) 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.\(^2\) 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+ 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

| Classification diagnosis (Group) | N | Age (year) | Fecal C. sinensis egg count (n/g) | HBs Ag (IU/ml) |
|---------------------------------|---|------------|---------------------------------|--------------|
| LC+ C. sinensis co-infection    | 18| 52.00 (38–68) | 200.00 (100–5000) | 405.00 (64.40–1432.00) |
| CHB+ C. sinensis 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) |
| C. sinensis 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).
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.24 Previous studies have found that serum T levels gradually elevated in male patients with CHB, compensated cirrhosis, and decompensated cirrhosis after hepatitis B.25 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.26 Moreover, Breidbart et al.27 have observed that treatment with T has no effects on the HBsAg levels in mice with testicular feminization mutation, while in normal

**FIGURE 1** Serum sex hormones levels in the HCs group, *C. sinensis* mono-infected, CHB mono-infected group, CHB+ *C. sinensis* co-infected, LC group, LC+ *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 ± interquartile range. Asterisks indicate statistically significant differences between Two groups, as measured by Kruskal-Wallis rank test (*p < .05, **p < .01, ***p < .001).
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+ C. sinensis patients, alterations of T may be a promising biomarker in co-infection. A previous study has indicated that C. sinensis co-infection affects the disease state and treatment response in HBV patients, 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, promotes 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 co-infection 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 coinfected 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 coinfected 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 coinfected HBV patients but also provide basis for further studies in this field.

AUTHOR CONTRIBUTIONS

Study design: BH. Information collection: WC. Sample assessment: 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.

THE LIMITATIONS OF THE STUDY

Our results not only highlight the importance of timely treatment and careful assessment of sex hormones for C. sinensis coinfected 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 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 co-infection 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 coinfected with C. sinensis.

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REFERENCES

1. Razavi-Shearer D, Gamkrelidze I, Nguyen MH, et al. Global prevalence, treatment, and prevention of hepatitis B virus
infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol.* 2018;3(6):383-403.

2. Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of hepatitis B virus infection and impact of vaccination on disease. *Clin Liver Dis.* 2016;20(4):607-628.

3. Höner Zu Siederdissen C, Cornberg M. The role of HBsAg levels in the current management of chronic HBV infection. *Ann Gastroenterol.* 2014;27(2):105-112.

4. Shin H, Oh J, Masurey E, et al. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci.* 2010;101(3):579-585.

5. Qian M, Utzinger J, Keiser J, Zhou XN. Clonorchiasis. *Lancet.* 2016;387(10020):800-810.

6. Tang Z, Huang Y, Yu X. Current status and perspectives of *Clonorchis sinensis* and clonorchiasis: epidemiology, pathogenesis, omics, prevention and control. *Infect Dis Poverty.* 2016;5(1):71.

7. Chai J, Darwin Murrell K, Lymbery AJ. Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol.* 2005;35(11):1233-1254.

8. Keiser J, Utzinger J. Food-borne Trematodiases. *Clin Microbiol Rev.* 2009;22(3):466-483.

9. Na BK, Pak JH, Hong SJ. *Clonorchis sinensis* and clonorchiasis. *Acta Trop.* 2020;203:105309.

10. Machicado C, Marcos LA. Carcinogenesis associated with parasites other than Schistosoma, opisthorchis and Clonorchis: a systematic review. *Int J Cancer.* 2016;138(12):2915-2921.

11. Papachristou GI, Schoedel KE, Ramanathan R, Rabinovitz M. *Clonorchis sinensis*-associated cholangiocarcinoma: a case report and review of the literature. *Digest Dis Sci.* 2005;50(11):2159-2162.

12. Qian M, Chen Y, Fang Y, et al. Disability weight of *Clonorchis sinensis* infection: captured from community study and model simulation. *PLoS Negl Trop Dis.* 2011;5(12):e1377.

13. Yan C, Wang L, Li B, et al. The expression dynamics of transforming growth factor-β/Smad signaling in the liver fibrosis experimentally caused by *Clonorchis sinensis*. * Parasit Vectors.* 2015;8:70.

14. Qian M, Chen Y, Liang S, Yang G, Zhou X. The global epidemiology of clonorchiasis and its relation with cholangiocarcinoma. *Infect Dis Poverty.* 2012;1(1):4.

15. Shen Y, Hou W, Yang Z, Xiao W. Hepatitis B virus infection and genotype in asymptomatic people from 10 ethnic groups in Yunnan, China. *World J Gastroenterol.* 2015;21(44):12586-12592.

16. Li W, Dong H, Huang Y, et al. *Clonorchis sinensis* co-infection could affect the disease state and treatment response of HBV patients. *PLoS Negl Trop Dis.* 2016;10(6):e0004806.

17. Nguyen HV, Mollison LC, Taylor TW, Chubb SAP, Yeap BB. Chronic hepatitis C infection and sex hormone levels: effect of disease severity and recombinant interferon-α therapy. *Intern Med J.* 2006;36(6):362-366.

18. Zietz B, Lock G, Plach B, et al. Dysfunction of the hypothalamic-pituitary-glandular axes and relation to child-Pugh classification in male patients with alcoholic and virus-related cirrhosis. *Eur J Gastroen Hepatol.* 2003;15(5):495-501.

19. Kuper H, Mantzoros C, Lagiou P, et al. Estrogens, testosterone and sex hormone binding globulin in relation to liver cancer in men. *Oncology.* 2001;60(4):355-360.

20. Yip TC, Wong GL, Chan HL, et al. Elevated testosterone increases risk of hepatocellular carcinoma in men with chronic hepatitis B and diabetes mellitus. *J Gastroen Hepatol.* 2020;35(12):2210-2219.

21. Abuzzi A, Fried B, Alikhan SB. Coinfection of Schistosoma species with hepatitis B or hepatitis C viruses. *Adv Parasitol.* 2016;91:111-231.

22. Kotepe KJ, Kotepui M. Prevalence of and risk factors for plasmodium spp. co-infection with hepatitis B virus: a systematic review and meta-analysis. *Malar J.* 2020;19(1):368.

23. Chen J, Xu M, Zhou D, et al. Canine and feline parasitic zoonoses in China. *Parasit Vectors.* 2012;5(1):152.

24. Vesper HW, Botelho JC, Wang Y. Challenges and improvements in testosterone and estradiol testing. *Asian J Androl.* 2014;16(2):178-184.

25. Serin A, Akarsu M, Akpinar H, Simsek I. Changes of some hormones levels in patients with hepatitis B virus-related chronic liver disease. *Gastroenterology Res.* 2013;6(4):134-138.

26. Tian Y, Kuo C, Chen W, Ou JJ. Enhancement of hepatitis B virus replication by androgen and its receptor in mice. *J Virol.* 2012;86(4):1904-1910.

27. Breidbart S, Burk RD, Saenger P. Hormonal regulation of hepatitis B virus gene expression: influence of androgen receptor. *Pediatr Res.* 1993;34(3):300-302.

28. Stroffolini T, Esvan R, Bilotti E, Sagnelli E, Gaeta GB, Almasio PL. Gender differences in chronic HBsAg carriers in Italy: evidence for the independent role of male sex in severity of liver disease. *J Med Virol.* 2015;87(11):1899-1903.

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