Journal of Helminthology

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The expression of sICAM-1 influenced by *Clonorchis sinensis* co-infection in CHB patients

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Research Paper

Cite this article: Qiu J, Shang M, Li W, Zhang H, Liao Y and Dong H (2024). The expression of sICAM-1 influenced by *Clonorchis sinensis* co-infection in CHB patients. *Journal of Helminthology*, **98**, e54, 1–6
https://doi.org/10.1017/S0022149X24000427.

Received: 13 March 2024
Revised: 27 May 2024
Accepted: 04 June 2024

Keywords:
sICAM-1; *Clonorchis sinensis*; hepatitis B virus; co-infection

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Abstract

Soluble Intercellular Adhesion Molecule-1 (sICAM-1) has emerged as an inflammatory biomarker of many essential functions. We investigated the level of sICAM-1 influenced by *Clonorchis sinensis* (*C. sinensis*) co-infection in chronic hepatitis B (CHB) patients to explore the degree of liver tissue inflammation and liver function damage after co-infection. The study included data from patients with *C. sinensis* mono-infection (n=27), hepatitis B virus (HBV) mono-infection (n=32), *C. sinensis* and HBV co-infection (n=24), post-hepatitis B liver cirrhosis (n=18), post-hepatitis B liver cirrhosis co-infected with *C. sinensis* (n=16), and healthy controls (n=39). The level of sICAM-1 was measured with specific enzyme-linked immunosorbent assay method. Compared to the healthy control group, all the experimental groups had significantly higher serum sICAM-1 levels. The levels of sICAM-1 in co-infected groups were significantly higher compared to the mono-infection groups and were positively correlated with the levels of glutamate aminotransferase (ALT) and aspartate aminotransferase (AST). Our research findings confirmed that co-infection could exacerbate liver tissue inflammation and liver function damage in patients, could raise the sICAM-1 level, and may lead to the chronicity of HBV infection. These results provide clues for pathological mechanism study and formulating treatment plans.

Introduction

Soluble Intercellular Adhesion Molecule-1 (sICAM-1) is a cell surface glycoprotein, which belongs to the immunoglobulin superfamily (Springer 1990). sICAM-1 expresses at a low basal level in immune, endothelial, and epithelial cells, but is upregulated in response to inflammatory stimulation (Hubbard and Rothlein 2000). Thus, sICAM-1 has emerged as a master regulator of many essential tissue functions both at the onset and the resolution of pathologic conditions (Philpott and Miner 2008; Wichert et al. 2017; Chu et al. 2023). sICAM-1 is generally not expressed on the surface of normal liver cells, but only weakly expressed on liver sinusoid endothelial cells, hepatocytes, Kupffer cells, and interstitial fibroblasts (Iwasawa et al. 2008). Previous studies have shown us the relationship between sICAM-1 and chronic hepatitis B (CHB). When infected with hepatitis B virus (HBV), sICAM-1 is strongly expressed on sinusoidal lining cells, mediating the interaction between immunocompetent cells to clear HBV. The level of sICAM-1 expression reflects the activity of hepatitis and the extent of liver tissue damage (Horiike et al. 1994; Nouri-Aria et al. 2019). It is closely associated with the occurrence, development, and prognosis of liver injury.

Hepatitis B virus (HBV) infection has been a long-standing global public health issue, with approximately 257 million chronic HBV-infected individuals worldwide and approximately 887,000 deaths from HBV related diseases each year (Nelson et al. 2016). According to the classification criteria of the World Health Organization, China is considered a country with moderate prevalence of HBV. There are approximately 70 million cases of chronic HBV infection in China, which is the main cause of viral hepatitis, cirrhosis, liver cancer, and liver failure (Shin et al. 2010; Honer Zu Siederdissen and Cornberg 2014; Li et al. 2023).

Clonorchiosis is a serious zoonotic parasitic disease caused by *Clonorchis sinensis* (*C. sinensis*) in the liver and bile ducts of humans and other mammals (Qian et al. 2016). About 12.5 million people in China are infected with *C. sinensis*, accounting for 85% of the global total number of infections. In 2020, the National Health Commission investigated the status of human parasitic infections, revealing that the prevalence of *C. sinensis* infection has increased by 75% compared to 1990 (Keiser and Utzinger 2009; Na et al. 2020). Chronic infection of *C. sinensis* can cause a series of liver and gallbladder diseases, such as liver fibrosis, gallstones, and common bile duct stones, and even lead to liver cirrhosis and liver and gallbladder cancer (Pak et al. 2019; Shang et al. 2020; Vale et al. 2020). The repeated infection of populations in epidemic areas can continuously promote the development of liver fibrosis, bringing a devastating disease burden to patients and society (Qian et al. 2011; Yan et al. 2015; Machicado and Marcos 2016).

Co-infection with *C. sinensis* and HBV is a phenomenon common in areas where *C. sinensis* is prevalent (Qian et al. 2012; Shen et al. 2015). Although the pathogenic mechanism of *C. sinensis* differs from that of HBV infection, both can cause damage to hepatocytes and speed up the process of the disease. At present, there is no clear consensus on whether *C. sinensis* will affect the infection of HBV. Studies have shown that the presence of *C. sinensis* can affect the anti-HBV treatment. In co-infected patients, the antiviral effect of simultaneous antiparasitic treatment is better than that of simple antiviral treatment (Li et al. 2016). However, in the clinical process of diagnosis and treatment, patients with *C. sinensis* were prone to be misdiagnosed. As mentioned earlier, the level of sICAM-1 expression can reflect the activity of hepatitis and the extent of liver tissue damage. In this study, we evaluated the level of sICAM-1 in patients co-infected with HBV and *C. sinensis*, and we explored whether it can indicate infection with *C. sinensis*, providing clues for further examination and treatment.

Materials and methods

Patients

This study included a total of 156 participants divided into six groups: patients with *C. sinensis* mono-infection (*C. sinensis*, n=27), patients with only HBV (CHB, n=32), chronic hepatitis B (CHB) patients co-infected with *C. sinensis* (CHB+*C. sinensis*, n=24), patients with single post-hepatitis B liver cirrhosis (LC, n=18), post-hepatitis B liver cirrhosis patients (LC) with *C. sinensis* co-infection (LC+*C. sinensis*, n=16), and healthy controls (HCs, n=39). The participants were selected from patients in the Third Affiliated Hospital of Sun Yat-Sen University.

The diagnosis in all subjects was based on clinical and laboratory data. The inclusion criteria for participants infected with *C. sinensis* were identification of eggs through microscopic exams for stool, excluding hepatitis B virus infection. As for participants infected with HBV, the inclusion criteria were HBV surface-antigen-positive and HBV DNA >10 IU/ml. The inclusion criteria for co-infected with *C. sinensis* and HBV were that *C. sinensis* eggs were found in the stool, as well as HBV infection. The inclusion criteria for chronic hepatitis B patients with liver cirrhosis was that the patients were clinically confirmed to have liver cirrhosis by liver biopsy and imaging tests after HBV infection. The inclusion criteria for patients with liver cirrhosis after co-infection were that *C. sinensis* eggs were found in the stool based on chronic hepatitis B with liver cirrhosis. The inclusion criteria for the healthy control group were negative for both HBV and *C. sinensis* as well as normal liver function indexes.

The participants were not included if they were under 18 years old or over 70 years old, pregnant, co-infected with other parasites and other types of the hepatitis virus, had liver diseases caused by metabolic disorders, drugs, and poisoning, acute infection, malignant tumor, human immunodeficiency virus (HIV) infection, thyroid dysfunction, autoimmune diseases, and so on.

The detection of sICAM-1 in serum

The serum was separated from peripheral blood of patients. The level of serum sICAM-1 was detected by enzyme-linked immunosorbent assay (ELISA) (Reagent kits purchased from Dakewe Biotech Co., Ltd.). First, 100μl diluted cytokine standard or 100μl diluted serum samples were added to each well. Second, 50μl biotinylated antibodies were added to all wells above. They were covered and incubated for one hour at room temperature. Then, the wells were washed three times by a washing buffer. Added 100μl diluted Streptavidin-HRP to each well and incubated all wells for twenty minutes at room temperature. The wells were washed again and incubated with 100μl TMB for 15 minutes before the addition of stop solution. Finally, the ELISA plate was determined at 450 nm with a correction wavelength of 630 nm.

The detection of liver function index

The level of aminotransferase (ALT) and aspartate aminotransferase (AST) in serum was detected by turbidimetric immunoassay using the Hitachi 7600 automated biochemical analyzer from Japan.

Statistical analysis

The data were analyzed by Prism 9.5.0 statistical software. The normally distributed data were presented as the mean±SD, and the skewness distributed data were presented as the median (IQR). The comparison between two groups was analyzed by the Mann-Whitney test. The comparison with more than two groups used the one-way ANOVA test or Kruskal-Wally's test. The correlation between two indexes was analyzed by Spearman's correlation analysis. $P<0.05$ was regarded as statistically significant.

Results

General information

General information of study participants is presented in Table 1, including age and levels of AST and ALT in all groups and results of both fecal *C. sinensis* egg count and HBV DNA copies in the patient groups. There were no significant differences in age among six groups and in fecal *C. sinensis* egg counts among the patient groups. All five infected groups of patients showed higher levels of AST and ALT than the healthy control subjects ($P<0.05$, respectively). The CHB+*C. sinensis* group had higher levels of AST and ALT than the CHB group ($P<0.05$). The LC+*C. sinensis* group had higher levels of AST than the LC group ($P<0.05$), while there was no difference in the ALT level.

The HBV DNA copies in the patient groups is shown in Figure 1. There were no significant differences between the CHB+*C. sinensis* and LC+*C. sinensis* groups, as well as the CHB and LC groups. The CHB+*C. sinensis* group had a significantly higher level of HBV DNA copies than the CHB group (a: $P<0.05$). Compared with the LC group, the LC+*C. sinensis* group had a significantly higher level of HBV DNA copies (b: $P<0.05$).

The comparison of the levels of sICAM-1 in all groups

As shown in Figure 2, compared with the healthy control group, the level of sICAM-1 significantly elevated in all the patient groups ($P<0.05$, respectively). In the co-infected groups, the CHB+*C. sinensis* group exhibited a significant elevation in sICAM-1 level than the CHB group ($P<0.05$), and the LC +*C. sinensis* group demonstrated a significant increase in sICAM-1 level compared with the LC group ($P<0.05$). However, there was no significant difference in sICAM-1 level between the CHB+*C. sinensis* and LC+*C. sinensis* group. In mono-infected

https://doi.org/10.1017/S0022149X24000427 Published online by Cambridge University Press

Table 1. Clinical statistics of the study subjects

Classification diagnosis (Group)	N	Age(year)	Fecal *C. sinensis* egg count (n/g)	HBV DNA copies, log	AST(U/L)	ALT(U/L)
CHB+*C. sinensis* co-infection	24	46.5(38–54)	100(100–200)	4.31(1.0–7.31)[b]	78(22–637)[a,b,c]	78(31–810)[a,b,c,d]
LC+*C. sinensis* co-infection	16	51.0(42–60)	100(100–1500)	3.66(1.57–7.36)[d]	97.5(28–1046)[a,b,c,d]	79.5(18–929)[a]
CHB (HBV mono-infection)	32	45.5(40–51)		3.08(1.15–8.70)	35.5(21–174)[a,d]	43(17–177)[a]
LC (HBV mono-infection)	18	50.2(42–58)		2.55(1.23–5.20)	59.9(15–176)[a,b]	37.5(20–181)[a]
C. sinensis mono-infection	27	46.9(35–57)	100(100–2000)		28(14–86)[a]	45(15–156)[a]
HCs	39	47.3(41–53)			20(15–27)	17(10–28)

Abbreviations: CHB: chronic hepatitis B; *C. sinensis: Clonorchis sinensis*; HCs: healthy controls; LC: liver cirrhosis.
[a]Statistically significantly different vs. healthy control subjects
[b]Statistically significantly different vs. CHB patients
[c]Statistically significantly different vs. *C. sinensis* patients
[d]Statistically significantly different vs. LC patients

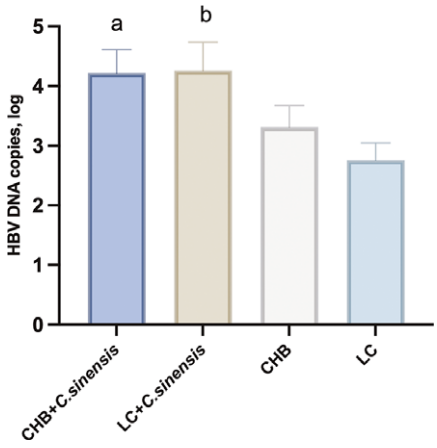

Figure 1. HBV DNA copies in patient groups. HBV DNA was detected by FQ-PCR and showed in log. Patients in CHB+*C. sinensis* group had higher HBV DNA copies than the CHB group (a: $P<0.05$). Patients in LC+*C. sinensis* group had higher HBV DNA copies than LC group (b: $P<0.05$). Analyzed by Mann-Whitney test.

groups, the LC group had a higher level of sICAM-1 than the CHB group ($P<0.05$).

The correlation analysis between the levels of sICAM-1 and AST, ALT in co-infected groups

The correlation analysis between the levels of sICAM-1 and AST, ALT is shown in Figure 3. Figure 3A shows a positive correlation between the level of sICAM-1 and AST in the CHB+*C. sinensis* group (Pearson $r=0.657$, $p<0.0001$). The correlation equation is Y = 16.31*X + 2839. Figure 3B shows a positive correlation between the level of sICAM-1 and ALT in the CHB+*C. sinensis* group (Pearson $r=0.556$, $p=0.0037$). The correlation equation is Y = 8.741*X + 31567. Figure 3C showed a positive correlation between the level of sICAM-1 and AST in the LC+*C. sinensis* group (Pearson $r=0.756$, $p=0.0039$). The correlation equation is Y = 9.624*X + 2877. Figure 3D showed a positive correlation between the level of sICAM-1 and ALT in the LC+*C. sinensis* group (Pearson $r=0.632$, $p=0.0021$). The correlation equation is Y = 11.31*X + 2747.

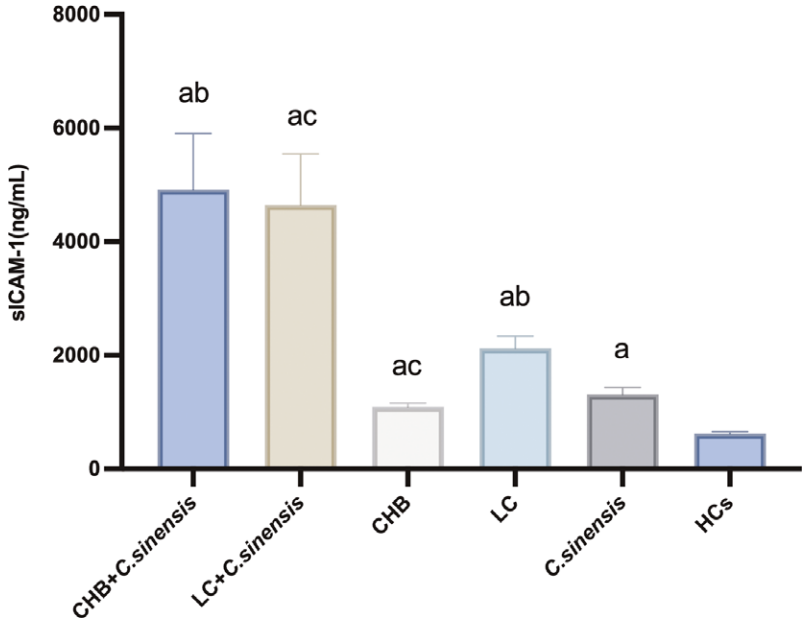

Figure 2. The levels of sICAM-1 in all groups. The level of sICAM-1 was detected by ELISA. All the patient groups had higher sICAM-1 levels than the healthy control group (a: $P<0.05$, compared with the HCs group). The CHB+*C. sinensis* and LC group exhibited a significant elevation in sICAM-1 level than CHB group (b: $P<0.05$, compared with the CHB group). The LC +*C. sinensis* group had higher sICAM-1 level compared with the LC group (c: $P<0.05$, compared with the LC group). Analyzed by Kruskal-Wally's test.

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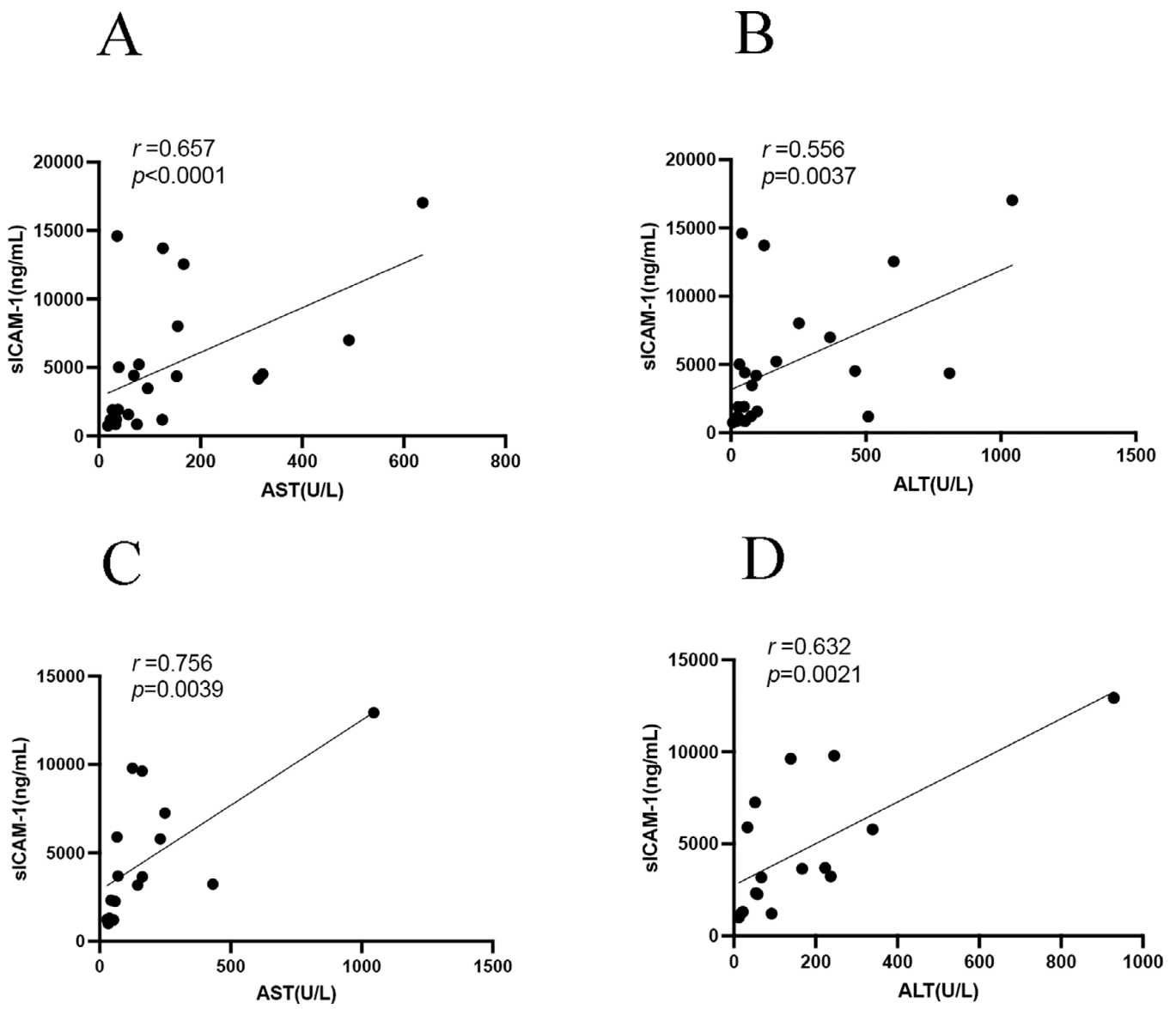

Figure 3. The correlation analysis between the levels of sICAM-1 and AST, ALT in CHB+*C. sinensis* and LC+*C. sinensis* group. There was a positive correlation between the levels of sICAM-1 and AST, ALT in the CHB+*C. sinensis* group (A and B) (r=0.657, 0.556, P<0.05) and the LC+*C. sinensis* group (C and D) (r=0.756, 0.632, P<0.05). Analyzed by Spearman's correlation analysis.

Discussion

This study found that co-infection could exacerbate liver tissue inflammation and liver function damage in patients, raising the sICAM-1 level and HBV DNA copies. Our data showed that compared with the healthy control group, the sICAM-1 levels in all the experimental groups were significantly increased(P<0.05). sICAM-1 is a single chain cell surface glycoprotein, which is usually not expressed on the surface of normal liver cells but is up-regulated in response to inflammatory stimulation. When infected with HBV, sICAM-1 is strongly expressed on sinusoidal lining cells, mediating the interaction between immune active cells to clear HBV (Hubbard and Rothlein 2000; Iwasawa et al. 2008; Nouri-Aria et al. 2019).

Our study also showed that the levels of sICAM-1 and HBV DNA copies in co-infected groups were significantly higher compared to the mono-infected groups. We consider that co-infection could exacerbate liver tissue inflammation in patients, raising the levels of sICAM-1 and HBV DNA copies. Previous studies have found that compared to patients infected with HBV, the expression of Th1 cytokines decreased in patients with co-infection, leading to a decline in the ability to clear HBV, while the expression of Th2 cytokines increased, exacerbating the persistent infection of HBV (Leung 1999; Henri et al. 2002; Dong et al. 2022). The combined action of proteins from HBV and *C. sinensis* can directly promote the activation of hepatic stellate cells, leading to the release of sICAM-1 (Hellerbrand et al. 1996; Li et al. 2016), which supports our findings.

Interestingly, compared with the CHB group, the levels of sICAM-1 and HBV DNA copies in the LC group increased significantly, while in the co-infected groups, there was no significant difference in the levels of sICAM-1 and HBV DNA copies. This suggests that co-infection may lead to the chronicity

https://doi.org/10.1017/S0022149X24000427 Published online by Cambridge University Press

of HBV infection. Our previous research results from *in vitro* experiments showed that the levels of pro-inflammatory cytokines in the co-infection group were lower than those in the HBV group, while the levels of liver fibrosis-related molecules were the opposite (Dong et al. 2022). This suggests that co-infection may exacerbate the development of liver fibrosis, leading to the chronicity of HBV infection. However, compared with the CHB group, the levels of sICAM-1 and HBV DNA copies in the CHB+*C. sinensis* group increased significantly. This indicates that the ability to clear HBV is not determined by a single cytokine, but the combined actions of various cytokines. As mentioned above, the decreased expression of Th1 cytokines and increased expression of Th2 cytokines exacerbated the persistent infection of HBV. Other research revealed that when *C. sinensis* infection occurs, secretion metabolic proteins (*Clonorchis sinensis* excretory secretory proteins, *Cs* ESP) are produced, among which the *Cs* Severin component has been reported to enhance the ability of IFN-α to clear HBV (Ahlenstiel et al. 2011; Edlich et al. 2012; Li et al. 2016). During co-infection, there is a more disrupted alteration in cytokine levels within the body. When the expression of cytokines that promote HBV infection is dominant, it is manifested as a persistent infection of HBV (Dong et al. 2022; Henri et al. 2002). Under the joint action of cytokines and macrophage inflammatory factors, sICAM-1 expression is directly or indirectly upregulated (Li et al. 2016). When the disease progresses to a certain extent, the levels of cytokines in the body tend to stabilize. The ability to clear HBV and promote HBV infection reaches a balance, leading to the chronicity of HBV infection. Therefore, the level of sICAM-1 did not increase significantly. We believe that sICAM-1 was closely related to the degree of liver function damage in patients with co-infection.

In this study, we also detected the levels of AST and ALT in each group. The levels of AST and ALT in the mono-infected groups were significantly higher than those in the healthy control group, but still within the normal range. Compared with the mono-infected groups, the levels of AST and ALT in the co-infected groups were significantly higher. ALT and AST are important transaminases in human body and mainly exist in hepatocytes. They are mainly used in clinical practice as evaluation indicators of liver function (Pol et al. 1991; Li et al. 2021). Some patients with HBV infection may be in the immune tolerance period or low replication period of the natural history of hepatitis B. There is only mild inflammation in the liver tissue, so the level of transaminase in the blood continues to be within the normal range (European Association for the Study of the Liver. Electronic address and European Association for the Study of the 2017). CHB patients with normal transaminase may also have varying degrees of inflammation, obvious fibrosis, and even cirrhosis (Tan et al. 2017). The clinical manifestations of *Clonorchiasis* can be acute or chronic depending on the degree of infection, duration, and immune status. Most infected individuals had normal serological indicators (Li et al. 2016). Co-infection of HBV and *C. sinensis* can aggravate the inflammation of liver tissue, leading to the increase of transaminase levels. Through the statistical analysis, there was a positive correlation between the levels of sICAM-1 and AST, ALT in the co-infected groups. This further confirmed that sICAM-1 could reflect the degree of liver inflammation after co-infection.

Research has demonstrated the involvement of sICAM-1 in the pathological damage mechanisms of chronic hepatitis B. It is closely related to the occurrence, progression, and prognosis of liver injury. We believe that during clinical diagnosis, a high level of sICAM-1 may alert doctors to the possibility of co-infection with *C. sinensis* in CHB patients, and sICAM-1 may serve as an indicator for monitoring treatment efficacy.

Abbreviations.

sICAM-1:	Soluble Intercellular Adhesion Molecule-1
HBV:	hepatitis B virus
C. sinensis:	*Clonorchis sinensis*
LC:	liver cirrhosis
CHB:	chronic hepatitis B
HCs:	healthy controls
AST:	Aspartate aminotransferase
ALT:	Alanine aminotransferase.

Author contribution. All the authors have agreed with the publication of this paper.
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Formal analysis: Mei Shang
Supervision: Yuan Liao
Writing: Jieru Qiu
All the authors participated in the critical revision and agreed to be responsible for all aspects of the work.
J. Qiu and M. Shang contributed equally to this work.

Data availability statement. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial support. This work was supported by grants from the National Natural Science Foundation of China (81902082).

Competing interest. The authors declare that they have no competing interests.

Ethical standard. The study was approved by The Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University ([2020]02-045-01)

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https://doi.org/10.1017/S0022149X24000427 Published online by Cambridge University Press