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Characteristics of Patients with Cryptogenic Cirrhosis: A Retrospective Study

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Backgrounds: This study aimed to achieve a better understanding of the characteristics of patients with cryptogenic cirrhosis (CC).
Methods: We retrospectively enrolled 50 patients with CC between January 2018 and December 2020 who were admitted to our hospital. Clinical data, biochemical and immunological parameters, viral markers, imaging findings and liver histopathological features of the patients were analyzed.
Results: The percentage of male patients with CC was 58% (29/50). The average age was 54 ± 17 years. Hepatitis C virus (HCV) IgG and hepatitis B surface antigen (HBsAg) were negative for all patients. Hepatitis B virus (HBV) DNA was tested in 68% (34/50) of the patients and the results were undetectable. Ceruloplasmin was detected in 96% (48/50) cases, while 10 cases were Kayser-fleischer ring negative. Immunological tests were conducted in 94% (47/50) of cases, antinuclear antibody (ANA) was elevated in eight cases, whereas anti-mitochondrial antibody (AMA) was elevated in three cases. Liver biopsy was conducted on 11 patients, of which seven were percutaneous and four were transjugular. Immunohistochemistry for HBsAg and HBcAg were all negative. Metavir scoring result showed that six of 11 patients had scores below G2S2.
Conclusions: The common laboratory tests especially noninvasive ones were conducted for most of the patients. Diagnosis of CC requires further detection to exclude specific diagnosis such as HBV DNA or intrahepatic covalently closed circular DNA (cccDNA) in HBcAb positive patients, genetic screening of Wilson's Disease in patients with low ceruloplasmin, etc.
Keywords: Cryptogenic cirrhosis; Characteristics; Liver biopsy; Retrospective study.

Introduction

Cryptogenic cirrhosis (CC) refers to cirrhosis whose etiology remains unclear after comprehensive evaluation[1]. CC accounts for about 5-30% of all liver cirrhosis[2, 3]. As an end-stage liver disease, CC severely threatens people's health worldwide and contributes to mortality and increasing orthotopic liver transplantation (OLT)[4, 5]. Therefore, in order to identify the underlying causes, it is necessary to screen the common causes of chronic liver disease before diagnosis, including viral hepatitis, autoimmune liver disease, alcoholic liver disease, drug-induced liver injury (DILI), vascular and biliary cirrhosis and genetic metabolic liver disease, which also requires invasive examination. However, due to thrombocytopenia and coagulation dysfunction in most patients with liver cirrhosis, percutaneous liver biopsy is risky, whereas transjugular liver biopsy is not commonly performed since it requires specific technology. Moreover, the reluctance of patients to accept an invasive examination is a drawback.

In addition to clinical diagnosis, the diagnosis of genetic metabolic diseases depends on gene sequencing, which is not common given its high cost. The above reasons lead to an obscure etiology of cirrhosis, which is clinically considered as CC. This study retrospectively analyzed the clinical characteristics of CC, in order to enhance the understanding of clinicians.

Methods

Patients

Fifty cases of CC admitted to the Third Affiliated Hospital of Sun Yat-sen University between January 2018 and December 2020 were retrospectively enrolled in the study. The diagnosis of liver cirrhosis conformed to the Chinese clinical guideline of liver cirrhosis[6], including: HBsAg negative, HCV IgG negative, excluding excessive drinking (more than five years of average alcohol consumption >40 g/day in males and >20 g/day in females), excluding autoimmune hepatitis with a score >13 points, ceruloplasmin <0.08 g/L, no history of special drug use. Informed consent was obtained from the patients to use the test results for medical research. This study conformed to the ethics principles of the Declaration of Helsinki, Good Clinical Practice and the regulatory requirements of China.

Basic data and laboratory tests

Demographic data of all patients were collected on admission. Blood routine test, biochemical tests, serological tests of viral hepatitis, autoimmune antibodies, and ceruloplasmin test were completed by the laboratory department. The detection of HBV

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DNA and HCV RNA was performed using Cobas TaqMan test by the liver disease laboratory. The abdominal color Doppler ultrasound was completed by the department of ultrasound. Liver biopsy, including percutaneous liver biopsy and transjugular liver biopsy, was performed by the doctors of the infectious disease department, ultrasound department and interventional vascular department.

Statistical analyses

Continuous variables were described as means and standard deviations (mean ± SD) or median. Categorical variables (e.g., age and gender) were described as numbers and percentages. All statistical analyses were performed using IBM SPSS Statistics Version 20 (Chicago, USA).

Results

General characteristics

The percentage of male patients with CC was 58% (29/50). The average age was 54 ± 17 years. Alcohol assumption (less than the standard of diagnosis of alcoholic liver disease) accounted for 20% (10/50). Four patients had a history of drug use including hypotensor and anti-diabetic agents. Sixteen patients were Child-Pugh A, 16 were B and 18 were C (Table 1).

Table 1. Demographic characteristics of CC patients (n = 50).

Characteristics	Mean (SD) or N (%)
Age, years	54 (17)
Gender, male, n (%)	29 (60.4%)
Alcohol consumption, n (%)	10 (20.4%)
Drug use, n (%)	4 (8.2%)
WBC, *10^9/L	4.62 (1.76)
HGB, g/L	107.9 (27.7)
PLT, *10^9/L	135.8 (82.9)
ALT, U/L	45.8 (54.8)
ALB, g/L	35.6 (7.3)
TB, μmol/L	63.3 (126.7)
PT, sec	16.3 (3.6)
INR	1.31 (0.39)
Child-Pugh A, n (%)	16 (32%)
Child-Pugh B, n (%)	16 (32%)
Child-Pugh C, n (%)	18 (36%)

WBC: white blood cell, HGB: hemoglobin, PLT: platelet, ALT: alanine aminotransferase, ALB: albumin, TB: total bilirubin, PT: prothrombin time, INR: international normalized ratio.

Serological test for viral hepatitis

All patients underwent serological test for viral hepatitis. Hepatitis C virus (HCV) IgG and hepatitis B surface antigen (HBsAg) were negative for all patients. HCV RNA was tested in 32% (16/50) of the patients whereas hepatitis B virus (HBV) DNA was tested in 68% (34/50) of the patients, and the results were undetectable. There were six types of serological results for HBV including three cases with all serological markers negative, eight with only hepatitis B surface antibody (HBsAb) positive, 16 with HBsAb, hepatitis B e antibody (HBeAb), hepatitis B core antibody (HBcAb) positive, 14 with HBsAb and HBcAb positive, eight with only HBcAb positive and one with HBcAb and HBeAb positive (Fig. 1). The positive rate for HBcAb was 66% (33/50). HBV DNA was tested in 24 of 33 (72.7%) HBcAb positive patients and the results were all negative (Fig. 2).

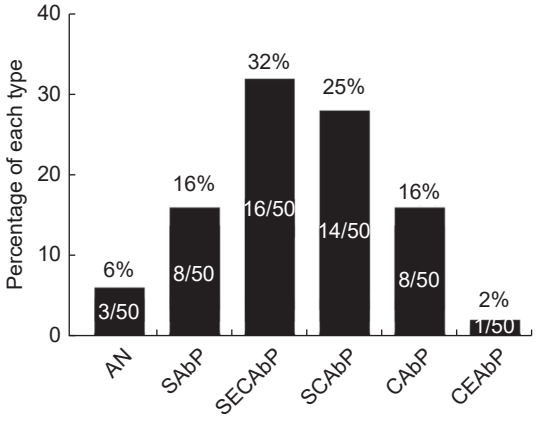

Fig. 1. Types of HBV serological results.
AN: All serum markers were negative. SAbP: HBsAb positive. SECAbP: HBsAb, HBeAb, HBcAb positive. SCAbP: HBsAb, HBcAb positive. CAbP: HBcAb positive. CEAbP: HBeAb, HBcAb positive.

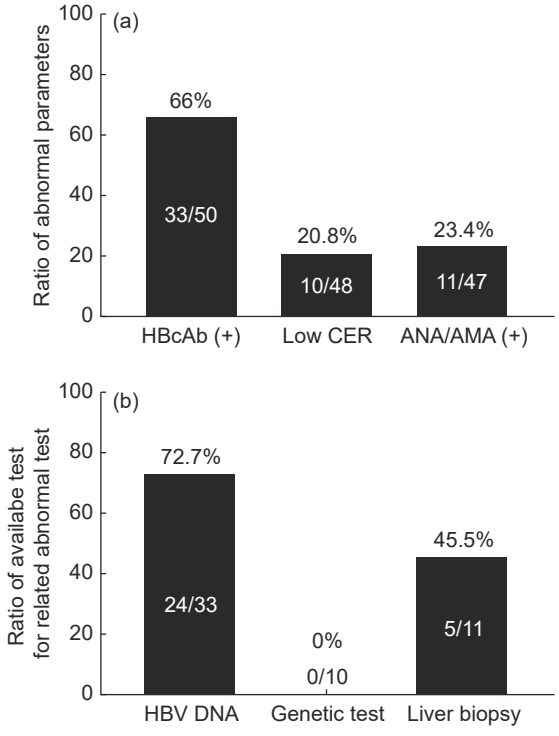

Fig. 2. Abnormal parameters and correlated further tests.
A. Ratio of abnormal parameters indicative of underlying etiology. HBcAb: hepatitis B core antibody, CER: ceruloplasmin, ANA: antinuclear antibody, AMA: anti-mitochondrial antibody.
B. Ratio of available tests for related abnormal tests.

Screening of Wilson's disease and autoimmune liver diseases

Ceruloplasmin was detected in 96% (48/50) of the cases and 10 cases showed a decline in whom Kayser-fleischer ring was negative. No genetic testing was performed on any patient. Immunological test was conducted in 94% (47/50) of the cases, antinuclear antibody (ANA) was elevated in eight cases whereas anti-mitochondrial antibody (AMA) was elevated in three cases. Among these 11 patients with positive ANA or AMA, only five patients underwent liver biopsy (Fig. 2).

Imaging and pathological findings

Ultrasound results showed 60% (28/47, three cases had splenectomy) of the cases had splenomegaly, with the average length of 136 ± 31.9 mm. Ascites was found in 36% (18/50) of the cases. Liver biopsy was conducted on 11 patients, of which seven were percutaneous and four were transjugular. Immunohistochemistry for HBsAg and HBcAg were all negative. No specific etiology was found among these specimens. Metavir scoring result showed that six of 11 patients had scores below G2S2 (Table 2).

Table 2. Pathological findings of 11 CC patients.

Patients	Gender	Age (yrs)	Procedure	Metavir	HBsAg	HBcAg
P1	Male	21	Percutaneously	G1S1	Negative	Negative
P2	Female	49	Percutaneously	G1S2	Negative	Negative
P3	Male	36	Percutaneously	G1S0	Negative	Negative
P4	Male	47	Percutaneously	G1S1	Negative	Negative
P5	Female	48	Percutaneously	G2S3	Negative	Negative
P6	Male	41	Percutaneously	G3S4	Negative	Negative
P7	Male	38	Percutaneously	G3S4	Negative	Negative
P8	Male	32	Transjugularly	G2S3	Negative	Negative
P9	Male	44	Transjugularly	G3S4	Negative	Negative
P10	Female	31	Transjugularly	G1S2	Negative	Negative
P11	Male	56	Transjugularly	G1S2	Negative	Negative

Discussion

For the diagnosis of CC, multiple measures have to be taken to exclude specific etiologies. However, due to the lack of broad knowledge of different etiologies among physicians and practical difficulties such as patient's reluctance and test cost, the assessment of such patients remains insufficient.

The majority of the common laboratory tests were conducted in CC patients in this study. Some of the results may provide clues to underlying etiologies. Serological tests for HBV among 50 CC patients showed that the positive rate of HBcAb was 66%, which implied that these patients were previously infected by HBV. Consequently, further tests should be carried out to rule out occult HBV infection (OBI). OBI refers to a status of negative serum HBsAg but positive serum or intrahepatic HBV DNA. The fundamental cause of OBI is the existence of covalently closed circular DNA (cccDNA) in the liver nucleus, which acts as a template to transcribe the corresponding mRNA[7]. OBI has important clinical significance. First, patients with OBI may deteriorate, or even progress to severe end-stage liver disease such as liver cirrhosis or liver cancer. In addition, these patients may experience HBV reactivation during immunosuppressive therapy[8]. In our study, serum HBV DNA was detected in 72.7% (24/33) of the HBcAb positive patients. None of these patients underwent tests for intrahepatic HBV DNA or cccDNA. These results suggested that OBI needs to be closely monitored.

Due to the low incidence of hereditary liver diseases, complicated clinical manifestations, lack of clinical understanding and limitations of test methods, CC has become a major problem for doctors. Many genetic disorders can lead to liver cirrhosis such as Wilson's disease and hereditary hemochromatosis[9, 10]. Genetic tests can be considered if abnormal metabolism of copper and iron exists. In our study, 10 patients had decreased ceruloplasmin, but urine copper or intrahepatic copper was not examined, and genetic tests were not conducted. Whole exome sequencing is valuable to assess whether genetic disorders exist in CC patients[11].

Autoimmune hepatitis (AIH) is an immune-mediated inflammatory liver disease, which is common in clinical settings. Multiple antibodies are related to AIH such as ANA. The diagnosis of AIH is based on a scoring system, which highlights histological evaluation[12]. Therefore, liver biopsy is necessary, especially if autoimmune antibodies are positive. Since percutaneous liver biopsy has high risk of bleeding, transjugular biopsy is a suitable option for patients with liver cirrhosis[13, 14]. However, due to its complicated process and high cost, it is not popular among clinicians and not acceptable to many patients. In our study, the ratio of liver biopsy was not high. Moreover, the fibrosis status of these patients was not consistent with their clinical manifestations, which might be due to the following reasons. First, patients who had undergone liver biopsy may be selected among patients who had less advanced liver cirrhosis. Second, sample error may exist. These results suggested that the diagnosis of liver cirrhosis needs comprehensive evaluation combining clinical features and histological changes.

This study had some limitations. First, this was a retrospective study, patients were treated by different doctors, the knowledge and understanding of CC may differ greatly. Second, we only enrolled patients with CC, no comparisons between CC and liver cirrhosis with definite etiologies were made, which may lead to inadequate perspective of CC. Finally, data related to metabolism such as body mass index (BMI), fasting blood glucose and

lipids were not analyzed. These aspects should be focused upon in the future to provide more insights into CC.

Conclusions

This study showed that the common laboratory tests especially noninvasive ones were conducted for the majority of CC patients. Diagnosis of CC requires further detection to exclude specific diagnosis such as HBV DNA or intrahepatic covalently closed circular DNA (cccDNA) in HBcAb-positive patients, genetic screening of Wilson's disease in patients with low ceruloplasmin, etc.

Abbreviations

AIH, autoimmune hepatitis; AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; CC, cryptogenic cirrhosis; cccDNA, covalently closed circular DNA; DILI, drug-induced liver injury; HBcAb, hepatitis B core antibody; HBeAb, hepatitis B e antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, Hepatitis B virus; HCV, Hepatitis C virus; OBI, occult HBV infection; OLT, orthotopic liver transplantation.

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Conflict of interest

The authors declare no editorial or financial conflict of interests.

Authors' contributions

WQG and YMC collected the data and wrote the manuscript. ZQW and YFZ performed the analysis. ZLG designed the study and revised the manuscript. YYM was responsible for the whole quality of the study and revised the manuscript. All authors have read and approved the submission of the manuscript.

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