The association of serum immunoglobulin and complement levels and liver fibrosis and inflammation stage in patients with chronic hepatitis B

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Research Article

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Abstract

Background: The utility of serum immunoglobulin and complement in chronic hepatitis B (CHB) patients remains controversial. The study aimed to investigate the association of serum immunoglobulin and complement levels and liver fibrosis and inflammation stage in CHB patients.

Methods:

A total of 720 patients with CHB who underwent liver biopsy were enrolled. Serum immunoglobulin and complement were measured before liver biopsy. And liver pathological results were recorded. Associations of serum immunoglobulin and complement levels and liver fibrosis and inflammation stage were analyzed.

Results:

C3, C4, IgG and IgG1 had statistical difference among different fibrosis groups and among different inflammation groups, whereas IgM and IgA had no statistical difference. Also, both C3 and C4 negatively correlated with fibrosis stage and inflammation stage. And both IgG and IgG1 positively correlated with fibrosis stage and inflammation stage. The area under curve (AUC) of IgG and IgG1for predicting fibrosis stage≥S2, ≥S3 and ≥S4 were 0.601 (95%CI: 0.556-0.647) and 0.647 (95%CI: 0.585-0.710), 0.615 (95%CI: 0.568-0.661) and 0.645 (95%CI: 0.570-0.719), and 0.657 (95%CI: 0.578-0.736) and 0.773 (95%CI: 0.656-0.890), respectively. The AUC of IgG and IgG1for predicting liver inflammation stage≥G2, ≥G3 and ≥G4 were 0.625 (95%CI: 0.567-0.682) and 0.658 (95%CI: 0.577-0.739), 0.628 (95%CI: 0.587-0.670) and 0.710 (95%CI: 0.651-0.769) and 0.659 (95%CI: 0.582-0.736) and 0.692 (95%CI: 0.538-0.847), respectively.

Conclusion: C3, C4, IgG and IgG1 were correlated with liver fibrosis and inflammation stage in CHB patients. IgG and IgG1 had diagnostic value for liver fibrosis and inflammation.

1. Introduction

Hepatitis B virus (HBV) infection is a serious public health problem. As estimated by the World Health Organization, 257 million people suffered from chronic hepatitis B (CHB) in 2015[1]. Long-term HBV infection easily leads to continuous damage of hepatocytes, resulting in liver fibrosis, cirrhosis, even hepatocellular carcinoma (HCC). As known, HBV infection has been the most common cause of HCC, being responsible for >50% of cases worldwide[2]. China is one of the highly endemic areas with a large number of patients infected with HBV[3]. Thus, it is important to monitor the changes of liver and to prevent HCC development in high-risk patients with advanced fibrosis. Liver biopsy is the golden standard for determining the liver fibrosis and inflammation stage. However, it is limited by several defects and to explore alternative indicators is essential.

Serum biomarkers are noninvasive and valuable for the management of CHB. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are most commonly used indicators to reflect liver

inflammation. AST-to-platelet (PLT) ratio index (APRI), fibrosis index based on the four factors (FIB-4), gamma-glutamyl transpeptidase (GGT)-to-PLT ratio (GPR) and other formulas are proposed to predict liver fibrosis[4-5]. However, serum immunoglobulin and complement levels are less often used to reflect liver inflammation and fibrosis stage. As known, they are usually used in autoimmune diseases. As for liver diseases, serum immunoglobulin is often thought to be elevated and serum complement is often thought to be reduced. The association between serum immunoglobulin and complement and pathological features in patients with CHB is rarely reported. Also, few prior studies have previously analyzed serum immunoglobulin G (IgG) subclass levels fully in patients with CHB. The clinical significance of serum immunoglobulin and complement in CHB remains controversial.

There is an urgent requirement for exploring the association between serum immunoglobulin and complement and pathological features in patients with CHB. Therefore, to further understanding, provide more evidence for the use of serum immunoglobulin and complement in patients with CHB, the present study retrospectively collected and analyze the clinical data of CHB patients who received liver biopsy and serum immunoglobulin and complement detection in recent years.

2. Materials And Methods

2.1 Study population

This study was a retrospective cohort study. Patients with CHB who were hospitalized for liver biopsy were recruited consecutively between January 2016 and December 2021 in the First Affiliated Hospital of Xiamen University. By consulting medical records, patients who had one of the following exclusion criteria were excluded. The exclusion criteria were as follows:(1) HBsAg positive no more than 6 mouths; (2) no serum complement C3 (C3), complement C4 (C4), IgG, immunoglobulin M (IgM) and immunoglobulin A (IgA) results within one week of liver biopsy; (3) co-infection with hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), or human immunodeficiency virus (HIV); (4) autoimmune liver disease, alcoholic liver disease, severe nonalcoholic fatty liver disease and other liver diseases; (5) with cancers; (6) autoimmune diseases (7) aged below 18 years or above 65 years; (8) incomplete clinical data. The study complied with the Helsinki Declaration and were approved by the Ethics Committee of the First Affiliated Hospital of Xiamen University.

2.2 Liver biopsy and laboratory test

Liver biopsies were performed using 16-G biopsy needles under the guidance of ultrasound. The pathological features of liver tissue including inflammation and fibrosi stage were evaluated by two independent pathologists in the Department of Pathology. When the pathologists differed in their assessments, an agreement was reached by discussion. The grading of inflammation and fibrosis was based on Scheuer scoring system: significant fibrosis was defined as greater than or equal to S2 (≥S2), severe fibrosis was defined as greater than or equal to S3(≥S3) and cirrhosis was defined as equal to S4[6].

Serum levels of IgG, IgM, IgA, C3, C4, IgG1, IgG2, IgG3, IgG4, ALT, AST, gamma-glutamyl transpeptidase (GGT), albumin (ALB), HBV-DNA and prothrombin time (PT) before liver biopsy were collected. Gengder, age and HBeAg status were also collected. Serum C3, C4, IgG, IgA, IgM, IgG1, IgG2, IgG3 and IgG4 were detected using immunoturbidimetry (BNII SYSTEM, Siemens). The reference ranges in the laboratory were as follows: C3: (0.9-1.8g/L), C4: (0.1-0.4g/L), IgG: (7-16g/L), IgM: (0.4-2.3g/L), IgA: (0.7-4.0g/L), IgG1: (4.05-10.11g/L), IgG2: (1.69-7.86g/L), IgG3: (0.11-0.85g/L) and IgG4: (0.03-2.01g/L).

2.4 Statistical analyses

The data analysis was performed using SPSS version 25.0 software and the Deepwise DxAI platform (http://dxonline.deepwise.com). Results are expressed as median (interquartile range, IQR) or number (percentage) where appropriate. Spearman's rank correlation coefficient analysis was used to analyze the associations between parameters and liver fibrosis and inflammation stage. ANOVA was used for normally distributed variables and the Kruskal-Wallis H test were used for non-normally distributed variables. The chi-square test was used for unordered categorical variables. ROC analysis were performed to evaluate the diagnostic accuracy of variables. $P<0.05$ was determined as significant.

3. Result

3.1 Patient characteristics

According to the inclusion and exclusion, a total of 720 CHB patients were eventually enrolled, including 498 male patients and 222 female patients. Average age was 34.99 years. HBeAg-positive rate was 58.75%. According to Scheuer scoring system, 205 patients were classified as S0-1, 310 as S2, 144 as S3 and 61 as S4, and 103 patients were classified as G0-1, 326 as G2, 238 as G3 and 43 as G4. The prevalence of significant fibrosis, severe fibrosis, and cirrhosis were 71.53%, 28.47% and 8.47%, respectively and the prevalence of inflammation stage≥G2, ≥G3 and ≥G4 were 85.69%, 40.42% and 7.36%, respectively. The age, gender, HBeAg-positive rate, ALT, AST, GGT, ALB, $\log_{10}$ HBV-DNA, PT were compared among different fibrosis stages and among different inflammation stages (Table 1). Age, ALT, AST, GGT, ALB and PT showed statistical difference among different fibrosis groups ($P<0.05$), whereas gender, HBeAg-positive rate and $\log_{10}$ HBV-DNA had no statistical difference ($P>0.05$). ALT, AST, GGT, ALB, PT, HBeAg-positive rate and $\log_{10}$ HBV-DNA showed statistical difference among different inflammation groups ($P<0.05$), whereas age and gender had no statistical difference ($P>0.05$).

3.2 Serum immunoglobulin and complement levels

As shown in Table 1, C3, C4 and IgG had statistical difference among different fibrosis groups ($P<0.05$), whereas IgM and IgA had no statistical difference among different fibrosis groups ($P>0.05$). Also, C3, C4 and IgG had statistical difference among different inflammation groups ($P<0.05$), whereas IgM and IgA had no statistical difference different inflammation groups ($P>0.05$). C3 and C4 decreased with the severity of fibrosis and inflammation. However, IgG increased with the severity of fibrosis and inflammation. 326 patients received IgG subclass detection. The 326 patients including 104 patients with

S0-1, 146 with S2, 55 with S3 and 21 with S4. And the 326 patients included 51 patients with G0-1, 162 with G2, 101 with G3 and 12 with G4. As shown in Table 1, only IgG1 had statistical difference among different inflammation groups (P<0.05) and among different inflammation groups (P<0.05). Also, IgG1 increased with the severity of fibrosis and inflammation.

Furthermore, the ratios of patients whose C3, C4, IgG and IgG1 were out of reference range were calculated (Table 2) . For all patients, serum C3 and C4 were reduced in 18.75% and 2.22%, respectively. And serum IgG and IgG1 were elevated in 17.08% and 28.22%, respectively. Only the ratios of elevated IgG1 showed statistic difference among different fibrosis stages (P<0.05) and among different inflammation stages (P<0.05).

3.3 Correlation analysis

As shown in Table 3, both C3 and C4 were negatively correlated with the liver fibrosis stage (C3: r=-0.19, P<0.05; C4: r=-0.21, P<0.05), and both IgG and IgG1 were positively correlated with the liver fibrosis stage (IgG: r=0.21, P<0.05; IgG1: r=0.28, P<0.05). Also, both C3 and C4 were negatively correlated with liver inflammation stage (C3: r=-0.24, P<0.05; C4: r=-0.24, P<0.05), and both IgG and IgG1 were positively correlated with liver inflammation stage (IgG: r=0.21, P<0.05; IgG1: r=0.36, P<0.05). In addition, IgM was positively correlated with liver inflammation stage (r=0.09, P<0.05). However, IgA, IgG2, IgG3 and IgG4 did not correlated with liver fibrosis stage and inflammation stage (P>0.05).

3.4 ROC analysis

To evaluate the accuracy of predicting liver pathological features, ROC analyses were performed. As shown in Table 4 and Figure 1, both IgG and IgG1 had statistical difference to predict significant fibrosis, severe fibrosis and cirrhosis, also had statistical difference to predict liver inflammation stage≥G2, ≥G3 and≥G4. The area under curve (AUC) of IgG and IgG1for predicting significant fibrosis, severe fibrosis and cirrhosis were 0.601 (95%CI: 0.556-0.647) and 0.647 (95%CI: 0.585-0.710), 0.615 (95%CI: 0.568-0.661) and 0.645 (95%CI: 0.570-0.719), and 0.657 (95%CI: 0.578-0.736) and 0.773 (95%CI: 0.656-0.890), respectively. The AUC of IgG and IgG1for predicting liver inflammation stage≥G2, ≥G3 and ≥G4 were 0.625 (95%CI: 0.567-0.682) and 0.658 (95%CI: 0.577-0.739), 0.628 (95%CI: 0.587-0.670) and 0.710 (95%CI: 0.651-0.769) and 0.659 (95%CI: 0.582-0.736) and 0.692 (95%CI: 0.538-0.847), respectively. However, both C3 and C4 had no statistical difference to predict liver pathological features. The AUC, optimized cut-off, sensitivity, specificity and Youden index were showed in Table 4.

4. Discussion

With the development of laboratory medicine, serum immunoglobulin and complement levels can be easily detected. More and more patients receive serum immunoglobulin and complement detection. To better understand the relationship between serum immunoglobulin and complement and liver fibrosis and inflammation stage in CHB patients based on clinical data is meaningful. In the present retrospective study, 720 CHB patients were enrolled according the inclusion and exclusion criteria. The prevalence of

significant fibrosis, severe fibrosis, and cirrhosis were consistent with the previous meta analysis[7]. The results revealed that serum immunoglobulin and complement were selectively correlated with liver fibrosis and inflammation stage in CHB patients.

The complement system plays an important role in both host innate immune defense and inflammatory progress of human diseases [8–13]. It is thought to be involved in the pathogenesis of multiple liver disorders[14]. In the present study, both C3 and C4 had statistic differences among different fibrosis groups and among different different inflammation groups. Also, correlation analysis revealed both C3 and C4 negatively correlated with fibrosis stage and inflammation stage. As known, liver is the major site for complement synthesis, accounting for up to 90% of the fluid-phase complement proteins [15–16]. On one hand, the damage of hepatocyte resulted in the decrease of complement synthesis; on the other hand, the complement activation resulted in the C3 and C4 cleavage. However, both C3 and C4 decreased in a small percentage (C3: 18.75%, C4: 2.22%). ROC analysis revealed that neither C3 nor C4 had no value in predicting liver fibrosis stage and inflammation stage. In this respect, it was difficult for clinicians to determine the liver fibrosis stage and inflammation stage depending on one time' result of serum C3 and C4. Continuous monitoring of serum C3 and C4 levels or specific reference ranges for CHB patients might be more significant.

Hypergammaglobulinaemia is a common finding (67%) in patients with cirrhosis[17]. As reported, immunoglobulins exerted a direct effect on hepatic fibrogenesis[18]. In the present study, only IgG had statistic differences among different fibrosis groups and among different different inflammation groups. Also, correlation analysis revealed that IgG positively correlated with fibrosis stage and inflammation stage, IgM positively correlated with inflammation stage with small correlation coefficient ($r = 0.09$) and IgA did not correlated with fibrosis stage and inflammation stage. Consistent with the previous study, there appears to be an association between serum IgG and extent of hepatic fibrosis in CHB patients[19]. Additionally, ROC analysis indicated that IgG had predictive value for liver fibrosis stage and inflammation stage with relatively low accuracy. IgG was a major antibody isotype in the blood that protected the body against pathogenic infection. As reported, IgG stimulates the proliferation of hepatic stellate cells and the expression of smooth muscle alpha-actin[18]. Thus, compared with serum IgA and IgM, serum IgG detection might be more important in the management of CHB. It was worth noting that only a small part of patients had evaluated IgG level (17.8%). Also, specific reference range of serum IgG for CHB patients was needed.

As known, the IgG family was comprised of IgG1, IgG2, IgG3 and IgG4[20]. But the association of IgG subclass and CHB was rarely reported. Thus, the IgG subclass was analyzed in the present study. Only IgG1 had statistic difference among different fibrosis stage and among different inflammation stage. Similar to IgG, IgG1 negatively correlated with fibrosis stage and inflammation stage. Also, ROC analysis revealed that IgG1 had predictive value for liver fibrosis stage and inflammation stage. Notably, the ratios of elevated IgG1 trended to increase with the fibrosis stages and and inflammation stages. These evidences suggested that IgG1was important in the management of CHB. IgG1 is the most abundant subclass in the serum, whilst IgG3 has the shortest half-life[21]. IgG4-related disease was reported for the

first time in 2014 in Japan[22]. Serum IgG4 was often used to exclude IgG4-related diseases. Zheng W's study indicated that certain serum IgG subclass levels were selectively increased or decreased depending of the type of liver disease, and IgG1/IgG level ratios in patients with viral liver disease were significantly increased [23]. Thus, more attention should be paid to IgG1 in CHB patient.

Our study has several limitations which ought to be acknowledged. Firstly, since this was a retrospective study, patient selection may be biased. More young patients might accept liver biopsy than older patients. Secondly, since this was an observational study, no follow-up process was performed. Finally, the number of samples was relatively small and not all patients received IgG subclass detection.

In conclusion, C3, C4, IgG and IgG1 were correlated with liver fibrosis and inflammation stage in CHB patients. IgG and IgG1 had diagnostic value for liver fibrosis and inflammation. Continuous monitoring of C3, C4, IgG and IgG1 levels or specific reference ranges for CHB patients might be more significant. With the development of machine learning, C3, C4, IgG and IgG1 might make contributions to predicting liver fibrosis and inflammation stage without liver biopsy. Further prospective longitudinal studies with a larger number of patients are needed.

Declarations

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Tables

Tables 1 to 4 are available in the Supplementary Files section

Figures

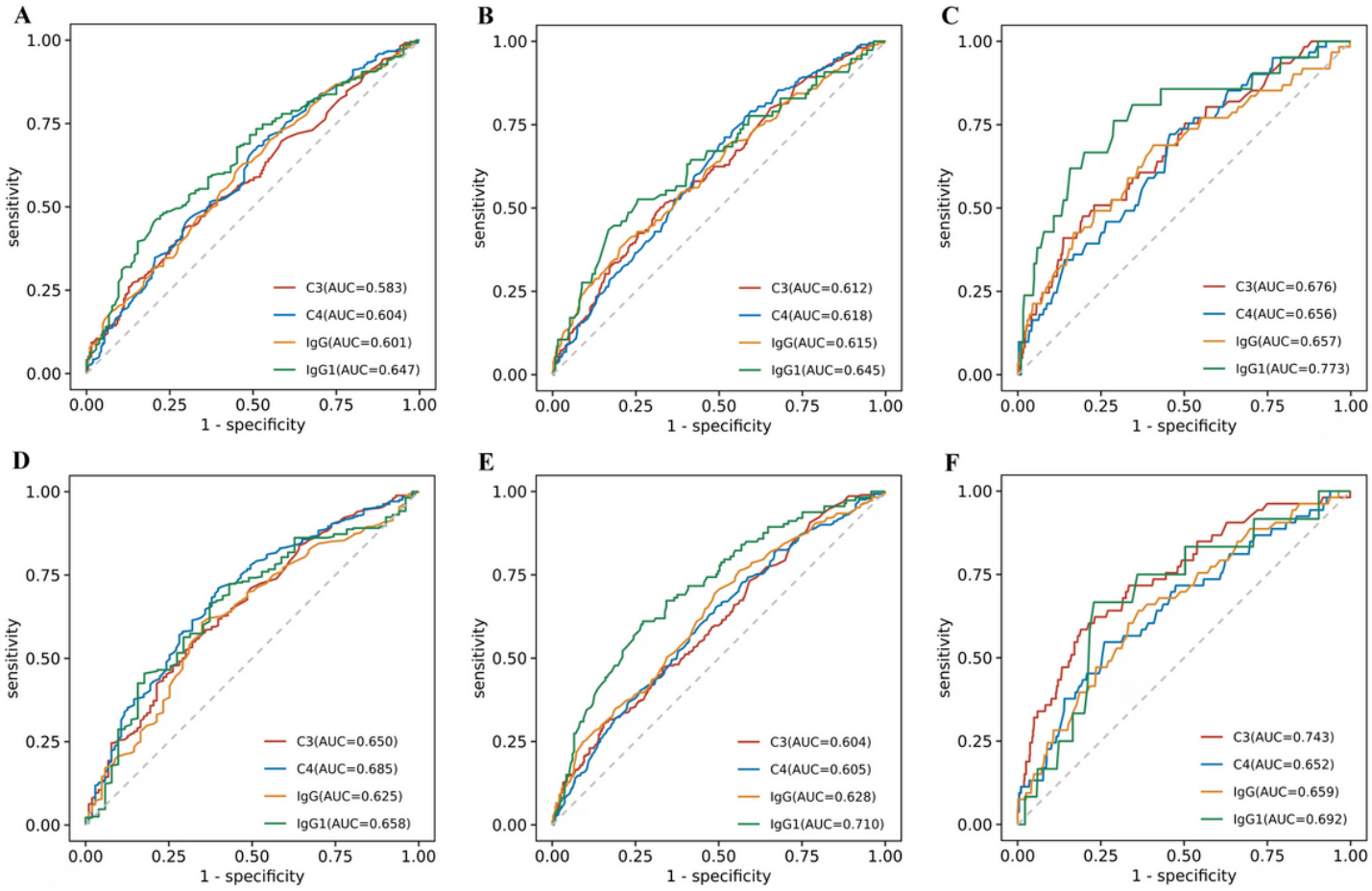

Figure 1

Receiver operating characteristic curve was constructed to predict liver pathological features. A, for predicting significant fibrosis, B, for predicting sever fibrosis, C, for predicting cirrhosis, D, for predicting

liver inflammation stage≥G2, E, for predicting liver inflammation stage≥G3, F, for predicting liver inflammation stage≥G4

Supplementary Files

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- Table1Baselinecharacteristicsofthepatients.docx
- Table2TheratiosofpatientswhoseC3.docx
- Table3Correlationanalysisbetweendifferentind.docx
- Table4Diagnosticaccuracyofdifferentindexesf.docx