Complement 3 could be available marker of liver function evaluation in patients with hepatitis B virus related cirrhosis and independent predictor of poor outcome in decompensated

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Research Article

Keywords: Complement, Cirrhosis, Liver Function, Infection, Mortality

Posted Date: June 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-654088/v1>

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Abstract

Aim

Hepatocytes biosynthesize about 80–90% of plasma complement proteins, including complement 3 (C3) and 4 (C4). The purpose of our study is to research the significance of complement in hepatitis B virus related cirrhosis (HBV-cirrhosis), discuss whether C3 and C4 could be served as available markers to assess liver function and prognostic in HBV-cirrhosis.

Methods

Blood samples were captured from 306 adult patients with HBV-cirrhosis, 23 chronic hepatitis B (CHB) without cirrhosis or liver failure, and 12 healthy adults. Serum C3 and C4 levels were compared in different groups, evaluating the correlation between C3/C4 levels and disease severity in patients with HBV-cirrhosis, analyzing the significance of complement components in infection and mortality of decompensated HBV-cirrhosis.

Results

The concentrations of C3 and C4 were markedly decreased in HBV-cirrhosis group than those in Chronic Hepatitis B (CHB) and healthy group ($p < 0.05$, respectively), especially in decompensated stage. In HBV-cirrhosis, The C3 and C4 levels were significantly negatively correlated with International Normalized Ratio (INR) and bilirubin, and significantly positively correlated with albumin, cholinesterase, cholesterol and platelet ($p < 0.05$, respectively). In addition, these were significance negative correlation with Child-Pugh score (C-P score), End-stage Liver Disease score (MELD score) and AST to platelet ratio ($p < 0.05$, respectively). More importantly, the C3 were significantly reduced in decompensated HBV-cirrhosis with infection or death, multivariate Cox-regression analysis revealed that lower C3 level was independent predictor of mortality in decompensated HBV-cirrhosis.

Conclusions

The C3 level could be served as available follow-up marker to evaluate liver function and disease severity in HBV-cirrhosis and superior to C4. Decreased C3 might be independent poor prognostic factor in patients with decompensated HBV-cirrhosis.

Introduction

Cirrhosis is end stage of chronic liver diseases caused by variety of etiological factor, the major is Hepatitis B virus (HBV) infection in Asian region, due to repeated inflammation-necrosis-regeneration process(1). Usually, it is classified as compensated and decompensated based on portal hypertension

and liver function, the decompensated cirrhosis is frequent and dangerous state causes numerous clinical contacts due to its complications, patients often die of multiple organ failure due to complicated ascites, gastrointestinal hemorrhage, hepatic encephalopathy, hepatorenal syndrome and sepsis(2, 3).

Prognosis of cirrhosis is influenced by competent clinical decision and selected clinical approach, precise liver function evaluation is essential for this process. The liver is multiple function organ fulfills the various metabolic requires of body, such as synthesizing proteins, producing digestive enzymes, and detoxifying various metabolites(4–6). Currently, liver function evaluation is based on the capacity of synthesizing albumin and clotting factors, as well as bilirubin metabolism. Several prognosis score systems, such as Child-Pugh score (C-P score) and End-stage Liver Disease score (MELD score), were broadly applied to clinical practice(4). Emerging views suggest that liver is considered a predominant immune organ and fulfill important immunologic function(7, 8). Hepatocytes account for up-to 70% of the liver cells, biosynthesize about 80–90% of plasma complement proteins(9). The complement as part of the immune system performs an important mission in physiological and pathological processes, such as microbial clearance, maintenance homeostasis, immune regulation, tissue regeneration and inflammation(10–12). The similar as activation of coagulation system, the complement system also through limited proteolytic steps result in generating protein-protein complexes. Three pathways, including the classical, the lectin and the alternative, serve for activation of the complement system, initiated through different molecules, converges at level of the complement 3 (C3)(13).

Number of research recognize that the etiopathogenesis of some liver disorders is contributed by the activation of complement system, involved in liver failure(14–16), viral hepatitis(17), alcoholic fatty liver disease(18), nonalcoholic fatty liver disease(19, 20), and autoimmune liver diseases(21). In nonalcoholic steatohepatitis, it was suggest that serum C3 level is closely correlation with the disease severity(20). Previous studies discussed the role of C3 in patients with HBV related acute-on-chronic liver failure and indicated that it can be independent risk factor for mortality(14, 15). A research in cirrhosis patients revealed that the level of lectin pathway complement components was decreased and closely related to disease severity, but did not investigate the role of C3 (22).

Despite the importance of complement system is currently turning increased attention by pathogenesis research of liver diseases, the significance in liver function test for cirrhosis is leaved out. Presently, more and more clinicians in practical decision-making ask for more well understanding and more precisely evaluating liver function to establish treatment strategy and improve outcome for patients with cirrhosis. Therefore, given the complements as the bridge between innate and adaptive immune systems and are produced by liver(13), we seek to explore its role in HBV-cirrhosis and its relationship with liver function and disease activity.

Methods

2.1 Patients and Sample Collection

In our study, we enrolled 306 adult patients with HBV related cirrhosis, they were inpatients who admitted to The Liver Disease Center of PLA, The 980th Hospital of PLA Joint Logistics Support Force, from January 2019 to January 2021. All patients were willing to participate. The exclusion criteria were: 1) the patient declined to be recruited in this study; 2) age less than 18 years old; 3) co-infected with hepatitis A, C, D, and E or HIV; 4) co-existence other liver disease, including steatosis hepatis, inherited metabolic liver disease, autoimmune liver diseases, cancer, biliary obstruction; 5) liver injury caused by drug intake and alcohol abuse; 6) presence history of rheumatic, renal, pancreas, endocrine, pulmonary, cardiovascular disease, splenectomy; 7) pregnancy.

The diagnosis of chronic HBV infection is based on Asian-Pacific clinical practice guidelines on the management of hepatitis B(1). The diagnosis of cirrhosis is based on [Assessment and management of cirrhosis in people older than 16 years: NICE guidance](#)(23). Cirrhosis was established by imageological diagnosis including computerized tomography scans or magnetic resonance image. We also enrolled 23 chronic CHB patients from our hospital. The CHB was defined as Hepatitis B surface antigen positive in serum more than 6 months and persistently repeatedly elevated alanine aminotransferase without others reasons(1). The CHB group patients were without cirrhosis and liver failure. The control group was consisting of 12 healthy adults. Morning fasting blood samples were collected after admission, then immediately detected and analyzed. The study was approved by the Ethical Committee of The 980th Hospital of PLA Joint Logistics Support Force (2018-KY-33), the written consent was obtained from each patient.

2.2 Patients Characteristics and Parameters

Detailed clinical data were captured including age, gender, decompensated event. The decompensated cirrhosis is defined as development of ascites, gastrointestinal hemorrhage, hepatic encephalopathy, hepatorenal syndrome and bacterial infection(2). Patient clinical parameters for evaluating disease severity involved routine blood tests, liver and renal function tests, coagulation function tests, cholesterol, Alpha-fetoprotein (AFP), HBV-DNA. Bacterial infection was identified by attending doctor according to patient clinical symptoms and infection index, such as routine blood tests, urine analysis, C-reactive protein (CRP), procalcitonin (PCT) and cultures (including blood, urine, sputum), in addition, take ascites analysis into account if ascites was present (including ascites culture)(2).

2.3 Disease Severity Evaluation

The liver-oriented scores, such as C-P score, MELD score and the [aspartate aminotransferase](#) (AST) to platelet ratio, were employed to evaluate severity of liver disease in this study. The C-P score and MELD score is calculated following the previously described criteria(4). The AST to platelet ratio is noninvasive tests for fibrosis and calculated as the ratio between the serum AST level and the platelet count(6).

2.4 C3, C4 Analysis

The serum levels of complement components C3 and C4 were detected by commercial kits following the manufacturer's instruction (Yilikang Biotech Co. Ltd, Zhejiang, P.R. China). Normal ranges of C3 and C4 is referred as 0.9 - 1.5 g/L and 0.2 - 0.4 g/L, respectively.

2.5 Statistical Analysis

The SPSS version 26 and GraphPad Prism 8 software were employed to perform the statistical analysis for all data. Variables were expressed as means (standard deviation, SD) or medians (interquartile range). Differences in categorical variables were compared by chi-squared test or Fisher's exact test. Differences in continuous variables were compared by non-parametric Mann-Whitney *U*-test or Kruskal-Wallis *H*-test. Spearman's rank correlation test was employed to analysis the relationship between two variables. The receiver operating characteristic (ROC) curve was applied for predicting the optimal cut-point of variable on infection and death. Cumulative probability of survival was calculated by Kaplan-Meier analysis and compared by the log-rank test. The correlation between clinical variables and infection or death was evaluated by univariate Logistic-regression or univariate Cox-regression analysis, respectively. The independent factors were assessed by Multivariate Logistic-regression or Cox-regression analysis with backward stepwise and likelihood ratio test. Two-tailed *p* value < 0.05 was considered to be statistically significant.

Result

3.1 Patients Characteristics

The baseline characteristics of total of 306 patients with HBV-cirrhosis, 23 CHB and 12 healthy adults were presented in Table 1. Gender and creatinine were not significant differences among three groups. Among 306 HBV-cirrhosis patients, 80 developed gastrointestinal hemorrhage, 51 developed hepatic encephalopathy, 122 developed ascites, 33 developed bacterial infection. Unfortunately, 20 patients with cirrhosis death. Thus, 190 patients were defined as decompensated cirrhosis, 116 patients were defined as compensated cirrhosis. There were no significant differences in gender, age, [alanine aminotransferase](#) (ALT), creatinine, AFP and HBV-DNA levels between compensated and decompensated cirrhosis, nevertheless, the International Normalized Ratio (INR), AST, albumin, bilirubin, cholinesterase, cholesterol, urea nitrogen, leukocyte, platelet levels and C-P, MELD score, AST to platelet ratio were significant differences.

3.2 Complement 3 and 4 levels markedly decreased in HBV-cirrhosis

The C3 and C4 levels were markedly decreased in HBV-cirrhosis group than those in CHB and control group (Fig1A, Fig1E). The median (interquartile range) concentration of C3 and C4 was 0.62 (0.48-0.83) g/L and 0.12 (0.09-0.1725) g/L in HBV-cirrhosis group, 1 (0.81-1.17) g/L and 0.18 (0.16-0.21) g/L in CHB, 1.115 (1.0825-1.2125) g/L and 0.23 (0.22-0.255) g/L in control, respectively. There were significant differences in three groups ($p<0.0001$).

We also investigated the serum C3 and C4 levels in cirrhosis sub-group, the result shown that decompensated cirrhosis group were significantly lower compared to compensated (Fig1A, Fig1E). The C3 was 0.865 (0.7225-1.0175) g/L and 0.52 (0.42-0.6325) in compensated and decompensated cirrhosis, the C4 was 0.17 (0.13-0.2175) g/L and 0.1 (0.07-0.13), both concentration were significant differences in two sub-group ($p<0.0001$). In spite of the C3 and C4 levels were reduced in compensated cirrhosis than CHB, there was no significant difference in C4 ($p=0.346$), while C3 level was significant difference ($p=0.042$). In addition, the concentration of C3 and C4 were markedly decreased in cirrhosis with gastrointestinal hemorrhage (Fig1B, Fig1F), hepatic encephalopathy (Fig1C, Fig1G) and ascites (Fig1D, Fig1H) than without. The median concentration of C3 in cirrhosis with gastrointestinal hemorrhage, hepatic encephalopathy and ascites were 0.535 (0.45-0.6475) g/L, 0.45 (0.37-0.54) g/L and 0.49 (0.3675-0.5825) g/L, were 0.68 (0.49-0.88) g/L, 0.67 (0.51-0.86) g/L and 0.765 (0.59-0.9275) g/L in without, respectively. With respect to C4, 0.1 (0.09-0.14) g/L in cirrhosis with gastrointestinal hemorrhage and 0.13 (0.09-0.19) g/L in without, 0.08 (0.06-0.11) g/L in cirrhosis with hepatic encephalopathy and 0.13 (0.1-0.18) g/L in without, 0.1 (0.07-0.13) g/L in cirrhosis with ascites and 0.15 (0.1-0.19) g/L in without. There were significance differences in all cirrhosis sub-groups ($p<0.05$). These results suggested that C3 and C4 might be play important role in disease advance of patient with HBV-cirrhosis.

3.3 C3 and C4 closely correlated with laboratory markers of liver function in HBV-cirrhosis

For investigating whether C3 and C4 could be used to assess liver function in HBV-cirrhosis, we analyzed the relationship between this complement components and laboratory markers of liver function. The correlation was presented in Table 2. The C3 and C4 shown significant negative correlation with INR (Fig.2A, Fig.3A), bilirubin (Fig.2C, Fig.3C), and significant positive correlation with albumin (Fig.2B, Fig.3B), cholinesterase (Fig.2D, Fig.3D), cholesterol (Fig.2E, Fig.3E), platelet (Fig.2F, Fig.3F). In addition, C3 and C4 shown slightly negative correlation with AST and urea nitrogen, slightly positive correlation with leukocyte. None of them were significant correlation with age, ALT, creatinine, AFP and HBV-DNA in HBV-cirrhosis.

3.4 C3 and C4 closely correlated with liver-oriented scores in HBV-cirrhosis

We also analyzed whether the levels of C3 and C4 were correlated with liver-oriented scores in HBV-cirrhosis. Firstly, we observed the levels of C3 and C4 in HBV-cirrhosis with different C-P score. Serum C3 level was significant lower in cirrhosis with C-P Score C compared with C-P Score A/B (Fig.4A), it was 0.835 (0.68-0.99) g/L in A, 0.56 (0.47-0.64) g/L in B, 0.42 (0.315-0.51) g/L in C. There was significant difference in three groups ($p<0.0001$), and significance negative correlation between C3 and C-P Score ($p<0.0001$). In terms of C4 (Fig.4D), it was 0.165 (0.13-0.2) g/L in A, 0.1 (0.08-0.13) g/L in B, 0.09 (0.06-0.11) g/L in C. In spite of C4 level was significance negative correlation with C-P Score ($p<0.0001$) and was significance difference between three C-P score groups ($p<0.0001$), there was no significant difference between B and C ($p=0.293$). Secondly, we observed the correlation between levels of C3/C4 and MELD score in HBV-cirrhosis. The median (interquartile range) of MELD score was 7 (6-9) and 12.5

(10-16) in compensated and decompensated cirrhosis, it was significant difference ($p<0.0001$). Spearman's rank correlation test revealed that both C3 and C4 were significance negative correlation with MELD score (Fig.4B, Fig.4E). In addition, we also observed the correlation between levels of C3/C4 and the AST to platelet ratio. The median (interquartile range) of AST to platelet ratio was 0.392 (0.22-0.678) and 0.78 (0.541-1.321) in compensated and decompensated cirrhosis, it was significant difference ($p<0.0001$). Similarly, C3 and C4 were significance negative correlation with AST to platelet ratio (Fig.4C Fig.4F). These results indicated that the C3 and C4 levels could be used to assess liver function in HBV-cirrhosis.

3.5 Serum C3 level markedly reduced in decompensated HBV-cirrhosis with bacterial infection

Next, we investigated the role of complement component in decompensated HBV-cirrhosis with bacterial infection. The results shown that C3 and C4 levels were lower in infection group than non-infection, the concentration of C3 was 0.37 (0.26-0.49) g/L in infection and 0.54 (0.45-0.65) g/L in non-infection, C4 was 0.08 (0.05-0.11) g/L in infection and 0.1 (0.08-0.135) g/L in non-infection, there were significance difference in two groups (Fig.5A, $p<0.0001$; Fig.5B, $p=0.002$; respectively). According to ROC curve (Fig.5C), the area under curve of C3 (AUC=0.817, 95%CI: 0.735-0.9, $p<0.0001$) was markedly higher than C4 (AUC=0.671, 95%CI: 0.564-0.779, $p=0.002$). These results suggested that C3 could be used to more well assess disease severity in HBV-cirrhosis compare with C4, the lower of C3 level, the higher bacterial infection risk in decompensated.

3.6 Serum C3 level was predictor of mortality in decompensated HBV-cirrhosis

We also explored the significance of complement components in decompensated HBV-cirrhosis patients who decease. The results shown that C3 and C4 levels were lower in death group than survival, the concentration of C3 was 0.335 (0.225-0.405) g/L in death and 0.53 (0.4475-0.64) g/L in survival, C4 was 0.075 (0.05-0.1075) g/L in death and 0.1 (0.08-0.13) g/L in survival, there were significance difference in two group (Fig. 5D, $p<0.0001$; Fig. 5E, $p=0.014$; respectively). According to ROC curve (Fig. 5F), the optimal cut-point of C3 level for predicting death was 0.415 g/L (sensitivity: 80%, specificity: 82.4%), the C4 level was 0.065 g/L (sensitivity: 45%, specificity: 84.1%), the area under curve of C3 (AUC=0.829, 95%CI: 0.725-0.933, $p<0.0001$) was markedly higher than C4 (AUC=0.669, 95%CI: 0.533-0.806, $p=0.013$).

Next, we observed whether the C3/C4 could be served as predictor of mortality in decompensated HBV-cirrhosis. 190 decompensated HBV-cirrhosis patients were grouped into 2 groups according to the optimal cut-point of C3 level (low grade: $C3 \leq 0.415$ g/L, $n = 46$; high grade: $C3 > 0.415$ g/L, $n = 144$). Similarly, based on the optimal cut-point of C4 level, patients with decompensated cirrhosis also were grouped into 2 groups (low grade: $C4 \leq 0.065$ g/L, $n = 36$; high grade: $C4 > 0.065$ g/L, $n = 154$). We analyzed the potential impact of different C3 or C4 levels on survival of decompensated HBV-cirrhosis, Kaplan-Meier analysis revealed that significantly worse survival rate in low C3 level grade than high C3 group (Fig. 5G, $p<0.0001$), high C4 level group were better in survival rate of decompensated cirrhosis than low C4 group (Fig. 5H, $p=0.001$).

In addition, assessment by univariate Cox-regression analysis were presented in Table 3. We showed that $C3 \leq 0.415$ g/L (HR: 15.194, 95%CI: 5.074-45.502, $P < 0.001$), $C4 \leq 0.065$ g/L (HR: 3.895, 95%CI: 1.613-9.406, $P = 0.003$), presence of hepatic encephalopathy (HR: 3.624, 95%CI: 1.501-8.748, $P = 0.004$), presence of ascites (HR: 11.449, 95%CI: 1.533-85.535, $P = 0.017$) and C-P Score C (HR: 12.824, 95%CI: 3.096-53.119, $P < 0.0001$) were significantly correlated with mortality in decompensated cirrhosis. Only lower C3 level (HR: 7.11, 95%CI: 2.25-22.467, $P = 0.001$) and C-P Score C (HR: 4.661, 95%CI: 1.021-21.286, $P = 0.047$) were independently hazardous factor for mortality. These results suggest that the C3 level could be served as predictor of mortality in decompensated HBV-cirrhosis.

Discussion

There were lots of researches explored the significance of complement in various liver disease, however, the linkage of complement protein to liver function is poorly alluded to in liver disease study let along in cirrhosis. With respect to liver function tests, many of tests is not to evaluate actual liver function but pinpoint damage source where may be taking place, such as aminotransferase and alkaline phosphatase, it is misguided(6, 13). The factual liver function based on a series of physiological function, including synthetic, secretion, excretory and immunity. Complement protein has not been applied as part of liver function tests to become follow-up marker. In our study, of the pivotal components of complement system, the C3 and C4 were systematically evaluated.

In patients with HBV-cirrhosis, we found that C3 and C4 levels were markedly decreased compared with CHB and health control, especially in decompensated stage, such as gastrointestinal hemorrhage, hepatic encephalopathy and ascites. Previous research pointed that the liver (mainly hepatocytes) biosynthesize about 90% of complement proteins, including C3 and C4. One rational explanation for lower C3 and C4 levels in cirrhosis is that decreased biosynthesis of hepatogenous proteins on account of impairing hepatocytes synthesize function. For verifying the hypothesis, we investigated the other laboratory markers which reflect hepatic synthetic function, including albumin and clotting factors(4, 5). Results shown that the significant correlations were existed between albumin or INR and C3/C4, which was highly consistent with disease severity. This significant correlation also were existed in serum cholinesterase(24) or total cholesterol(25), which were hepatocyte synthesize. These results supported that the C3 and C4 could be server as biochemical markers to assess hepatocytes synthesize function in cirrhosis.

Total bilirubin is considered as important biochemical marker of liver function and included in C-P score or MELD score systems(4, 5), our results shown that C3 and C4 levels were significant correlation with total bilirubin. The C-P score or MELD score were composite scores systems and widespread applied to assess liver function and severity of liver disease. There were significant negative correlations between C3 or C4 levels and C-P score or MELD score. However, unlike serum C3 level were gradually decreased following C-P score A up-to C, C4 level was no significant differences between C-P Score B and C. Therefore, we suggested that C3 might be a potential biochemical marker to evaluate severity of HBV-

cirrhosis and superior to C4, the lower of C3 level, the more severely of disease and deteriorated liver function.

The decreased platelet count is generally regarded as suggestive marker of cirrhosis, which is consistent with advancing fibrosis and cirrhosis(6). As fibrosis progresses, the AST to platelet ratio is gradually elevated(6). Some previous research pointed that complement system is contributed to pathogenesis of liver fibrosis(9, 26, 27). Our study revealed that the declined level of C3 and C4 were consistent with the decreased platelet count in cirrhosis, and there was significant correlation with the AST to platelet ratio. Intriguingly, the serum C3 level was significant difference between compensated cirrhosis and CHB, this phenomenon cannot be observed in C4, albumin, INR and total bilirubin but in leukocyte and platelet levels. Thus, we hypothesize that C3 might be potential noninvasive suggestive biochemical markers of predict cirrhosis, it is worthy of further exploration because of very important for improving prognosis of patients with chronic HBV infection. There was study reported that HBV could inhibit the synthesis and expression of C3 and C4 in vivo or in vitro(28). However, we did not observe the correlation between HBV-DNA level and C3 or C4 level in cirrhosis, the widespread antiviral therapy for HBV-cirrhosis patient might be reasonable explanation for this inconsistent phenomenon, the further clarification is necessary.

Emerging views suggest that liver is considered a predominant immune organ and fulfill important immunologic function(7, 8). The liver not only enrich a great deal of immune cells but also biosynthesize most of complement components and pathogen-recognition receptors, it has profound implications on innate immunity and adaptive immunity. With progresses of cirrhosis and worsening of liver function, innate and adaptive immune dysfunction are inevitable outcome, enhanced infection risk including systemic bacterial infections and bacterial translocation has significant impact on patient consequence(29, 30). Complement system play a bridging role of innate and adaptive immunity, cascaded activation through classical, lectin pathway or alternative pathways, the C3 takes center stage inside this process(13). Number of previous researches mentioned that lower complement level could enhance the sensibility to bacterial infection in cirrhosis patient due to weaken microbes recognition and clearance capacity(22, 31).

In our decompensated HBV-cirrhosis patient population, the serum C3 levels was remarkably decreased in those subjects with infection, and superior to C4 in predicting infection according to ROC curve. Previous study on patients with alcoholic cirrhosis indicated that acquired C3 deficiency predispose to infection(31), which is line with our research. The role of complement component not only involved in extracellular but also in intracellular or subcellular space(10, 12). One study on patients with cirrhosis revealed that decreased lysozyme and C3 levels in alveolar lining components induce deficiency of bactericidal activity is contributed to higher sensitivity to *Streptococcus Pneumoniae*(32). The other study in gut epithelial cells clarified that complement play dramatic role in maintaining homeostasis of host/environmental mucosal interface(33, 34). The most common infection in cirrhosis is Spontaneous bacterial peritonitis(35, 36), the homeostasis in gut epithelial cells is broken by defect of complement component induce bacterial translocation may be a probable reason, it is worth to further explore. Patient with cirrhosis occur infection generally result in higher mortality, early identification of high risk factors

for bacterial infection episodes are paramount importance. Presently, only liver disease severity and gastrointestinal hemorrhage are considered as important clinical predictors(36), our study suggested that lower C3 level might be another, which could be considered as an available biochemical marker to evaluate liver immunologic function.

In addition, we also revealed that the lower C3 and C4 levels were associated the worse survival rate in decompensated HBV-cirrhosis patient population, and C3 might be an independent prediction factor of the mortality. Deteriorated liver function in advanced cirrhosis might be sound reason for this phenomenon. The serum C3 and C4 levels is maintained by biosynthesis and activation consumption(11). The activation of complement system maintains subtle balance between pro-inflammatory and anti-inflammatory, it is a double-edged sword in disease progress. On the one hand, activation could result in liver tissue damage and amplify systemic inflammatory. Evidence that membrane attack complex could be found to deposit at liver parenchyma of patient with fulminant hepatitis or acute liver failure(16, 37, 38). In rat model, soluble complement receptor 1 could be administrated to efficiently inhibit C3 activation result in ameliorating hepatic ischemia–reperfusion injury induced liver injury(39, 40). In primate sepsis model, inhibiting C3 activation could attenuate *Escherichia coli* induced liver inflammatory injury(41). More importantly, C3 deficiency mice had been shown the lower systemic inflammation protect against fulminant liver failure compare with wild-type(42). These researches imply that excessive activation of complement might be a part reason in higher mortality of decompensated HBV-cirrhosis patients. Unfortunately, activation markers were not being quantified, it is limitation in our study. On the other hand, complement activation is advantageous for liver regeneration and required for hepatocyte survival(43). Number of studies had been shown the diminished ability of liver regeneration and exacerbated pathological changes in C3-deficient or/and C5-deficient mice than wild(44–46). One study on liver regeneration after hepatectomy confirmed that C3 play pivotal role in this process(47). It is might be the other reason of higher mortality in decompensated HBV-cirrhosis patients with lower C3 level, and worth to further explore. Taken together, our study suggested that C3 level might be a potential predictor of mortality in decompensated HBV-cirrhosis in admission.

Conclusions

In conclusion, our study shown that the serum levels of C3 and C4 are markedly decreased in HBV-cirrhosis, particularly in decompensated stage, and closely correlation with disease severity. The C3 level could be served as available follow-up marker to evaluate liver function, including synthetic and immunity, superior to C4, the lower of C3 level suggest the higher bacterial infection risk in decompensated cirrhosis. Furthermore, decreased C3 level might be independent predictor of mortality in decompensated HBV-cirrhosis. These results might assist clinician more well understanding and more precisely evaluating liver function to establish better treatment strategy and improve outcome for patients with cirrhosis when practical decision-making. The detailed role of the complement component in etiopathogenesis of HBV-cirrhosis urgently need to be sufficiently clarified.

List Of Abbreviations

HBV: Hepatitis B virus; HBV-cirrhosis: Hepatitis B virus related cirrhosis; CHB: Chronic hepatitis B; C3: Complement 3; C4: Complement 4; INR: International Normalized Ratio; C-P score: Child-Pugh score; MELD score: End-stage Liver Disease score.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of The 980th Hospital of PLA Joint Logistics Support Force (2018-KY-33). The written consent was obtained from each patient.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Identifying/confidential patient data however will not be shared.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was Supported by grants from the National Natural Science Foundation of China (81672041, URL: <http://isisn.nsfc.gov.cn/egrantweb/>).

Authors' contributions

JR and DXS contributed to the study idea; JR, JL and HFZ contributed to clinical data collection; JR contributed to statistical analysis and manuscript writing. All authors read and approve the final manuscript.

Acknowledgments

We thank Professor Rixiang Li from the Shaanxi University of Chinese Medicine affiliated Hospital for helpful supporting of our study.

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Tables

Table1 The baseline characteristics of HBV-cirrhosis patients, CHB patients and healthy control group.

Variables		Healthy control	Chronic hepatitis B	Compensated cirrhosis	Decompensated cirrhosis	P^I	P^{II}
No.		12	23	116	190		
Gender[male/female]		5/7	15/8	74/42	130/60	0.409	
Age (years)		45 (36.75-53.5)	49 (35-56)	55 (47-65)	56 (48-66)	<0.0001	0.286
INR		0.95 (0.8725-0.1005)	0.95 (0.89-1.11)	1.02 (0.96-1.11)	1.32 (1.19-1.5)	<0.0001	<0.0001
ALT (U/L)		20 (15.25-29.25)	96 (63-201)	25 (17.25-37.75)	24.5 (16-40.25)	<0.0001	0.9713
AST (U/L)		21.5 (18.25-25.5)	63 (47-155)	30 (23-39)	36 (26-62.25)	<0.0001	0.0003
Albumin (g/L)		45.6 (41.825-50.6)	40 (37.9-43.2)	41.2 (37.8-45.2)	29.7 (26.8-33.425)	<0.0001	<0.0001
Bilirubin (μ mol/L)		13.3 (10.45-19.2)	17.8 (12.9-34)	18.15 (13.125-27.775)	30.85 (19.15-51.55)	<0.0001	<0.0001
Cholinesterase (U/L)		8412 (7299.75-9850.75)	7125 (5683-8348)	6126 (4711-7845.75)	2843 (2002-3896.25)	<0.0001	<0.0001
Cholesterol (mmol/L)		4.195 (3.795-5.015)	4.22 (3.22-5.17)	3.73 (3.2525-4.225)	2.68 (2.1475-3.3025)	<0.0001	<0.0001
Creatinine (μ mol/L)		55 (48.25-63)	51 (41-59)	59 (47-66.75)	58 (47-76.25)	0.277	
Urea nitrogen (mmol/L)		4.31 (3.55-4.8875)	4.71 (3.96-5.31)	4.86 (4.225-5.85)	5.625 (4.245-9.365)	<0.0001	0.0008
AFP (ng/ml)		3.005 (2.3325-4.08)	6.67 (4.08-12.39)	3.325 (1.9825-6.1925)	2.625 (1.68-7.8175)	0.015	0.2998
Leukocyte (10^9 /L)		4.675 (4.5225-6.3175)	5.34 (4.74-6.75)	3.865 (3.07-5.26)	3.33 (2.32-5.1125)	<0.0001	0.0323
Platelet (10^9 /L)		146 (120.25-163.75)	177 (157-233)	77.5 (54-116.5)	47.5 (35-73)	<0.0001	<0.0001
HBV-DNA(log10 IU/ml)		N.D	4.58 $\pm$ 2.414	2.74 $\pm$ 1.567	2.5 $\pm$ 1.355	<0.0001	0.460
Gastrointestinal hemorrhage		N	N	N	80 (42.11%)	N.D	
Hepatic encephalopathy		N	N	N	51 (26.84%)	N.D	
Ascites		N	N	N	122 (64.21%)	N.D	
Infection		N	N	N	33 (17.37%)	N.D	
C-P Score	A	N.D	N.D	111 (95.69%)	29 (15.26%)	N.D	<0.0001
	B	N.D	N.D	6 (5.17%)	87 (45.79%)	N.D	
	C	N.D	N.D	0	73 (38.42%)	N.D	
MELD Score		N.D	N.D	7 (6-9)	12.5 (10-16)	N.D	<0.0001
AST/platelet ratio		N.D	N.D	0.392 (0.22-0.678)	0.78 (0.541-1.321)	N.D	<0.0001

Variables are shown as means (standard deviation, SD) or medians (interquartile range).

$P^{\square}$ the difference in variables between HBV-cirrhosis patients, CHB patients and healthy adults control group.

$P^{\square\square}$, the difference in variables between compensated and decompensated HBV-cirrhosis.

P -values were calculated by chi-squared test or Fisher's exact test, and non-parametric Mann-Whitney U -test or Kruskal-Wallis H -test.

INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; C-P Score, Child-Pugh Score; MELD Score, Model of End-stage Liver Disease score; AST/platelet ratio, AST to platelet ratio; N.D., not determined; N, no.

Table2 Non-parametric correlation between levels of C3 or C4 and characteristic of HBV-cirrhosis patients.

Variables	C3		C4	
	Spearman's rho	p-value	Spearman's rho	p-value
Age	-0.032	0.581	0.079	0.168
INR	-0.815	0.000	-0.682	0.000
ALT	-0.054	0.349	-0.017	0.761
AST	-0.205	0.000	-0.166	0.004
Albumin	0.713	0.000	0.57	0.000
Bilirubin	-0.521	0.000	-0.388	0.000
Cholinesterase	0.742	0.000	0.547	0.000
Cholesterol	0.615	0.000	0.446	0.000
Creatinine	-0.023	0.689	0.068	0.232
Urea nitrogen	-0.222	0.000	-0.123	0.032
AFP	-0.05	0.38	-0.05	0.382
Leukocyte	0.169	0.003	0.262	0.000
Platelet	0.446	0.000	0.496	0.000
HBV-DNA	-0.028	0.622	0.009	0.870
C-P score	-0.738	0.000	-0.543	0.000
MELD score	-0.756	0.000	-0.605	0.000
AST/platelet	-0.507	0.000	-0.471	0.000

Spearman's rank correlation test was employed to analysis the relationship between two variables.

INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; C-P Score, Child-Pugh Score; MELD Score, Model of End-stage Liver Disease score; AST/platelet ratio, AST to platelet ratio.

Table3 Analysis for the association of clinical factors with mortality in Decompensated HBV-cirrhosis.

Variate		No.	Cox-regression analysis			
			Univariate analysis		Multivariate analysis	
			HR (95%CI)	P-value	HR (95%CI)	P-value
Age		190	0.994 (0.959-1.031)	0.763		
Gender[male/female]		130/60	1.809 (0.750-4.366)	0.187		
C3 (g/L)	C3 > 0.415	144	15.194 (5.074-45.502)	<0.0001	7.110 (2.25-22.467)	0.001
	C3 ≤ 0.415	46				
C4 (g/L)	C4 > 0.065	154	3.895 (1.613-9.406)	0.003		
	C4 ≤ 0.065	36				
Gastrointestinal hemorrhage	N	110	0.908 (0.371-2.222)	0.833		
	P	80				
Hepatic encephalopathy	N	139	3.624 (1.501-8.748)	0.004		
	P	51				
Ascites	N	68	11.449 (1.533-85.535)	0.017		
	P	122				
C-P Score	A	30	12.824 (3.096-53.119)	<0.0001	4.661 (1.021-21.286)	0.047
	B	87				
	C	73				

Univariate and multivariate Cox-regression analysis for the association of factors with mortality in Decompensated HBV-cirrhosis.

HR, hazard ratio; CI, confidence interval; P, present; N, no.

Figures

Figure 1

Complement 3 and 4 levels in difference groups. (A, E) The concentrations of C3 and C4 were significantly lower in HBV-cirrhosis group than those in CHB group or control group ($p<0.0001$); Decompensated cirrhosis group were significantly lower compared to compensated ($p<0.0001$); C3 level was significant difference between compensated cirrhosis than CHB ($p=0.042$), while C4 level was no significant difference ($p=0.346$). (B, C, D) C3 level was significantly lower in cirrhosis with gastrointestinal hemorrhage ($p<0.0001$), hepatic encephalopathy ($p<0.0001$) and ascites ($p<0.0001$) than cirrhosis without. (F, G, H) C4 level was significantly lower in cirrhosis with gastrointestinal hemorrhage ($p=0.006$), hepatic encephalopathy ($p<0.0001$) and ascites ($p<0.0001$) than cirrhosis without. Con., Healthy control; CHB, Chronic hepatitis B; Cor., HBV-Cirrhosis; Com., Compensated cirrhosis; Dec., Decompensated cirrhosis; GH, Gastrointestinal hemorrhage; HE, Hepatic encephalopathy; AS, Ascites; P, present; N, no.

Figure 2

Correlation between C3 and laboratory markers of HBV-cirrhosis. (A) The C3 showed significant negative correlation with INR; (B), The C3 showed significant positive correlation with albumin; (C) The C3 showed significant negative correlation with bilirubin; (D) The C3 showed significant positive correlation with cholinesterase; (E) The C3 showed significant positive correlation with cholesterol; (F) The C3 showed significant positive correlation with platelet. INR, International Normalized Ratio.

Figure 3

Correlation between C4 and laboratory markers of HBV-cirrhosis. (A) The C4 showed significant negative correlation with INR; (B) The C4 showed significant positive correlation with albumin; (C) The C4 showed significant negative correlation with bilirubin; (D) The C4 showed significant positive correlation with cholinesterase; (E) The C4 showed significant positive correlation with cholesterol; (F) The C4 showed significant positive correlation with platelet. INR, International Normalized Ratio.

Figure 4

Correlation of C3 and C4 with liver-oriented score systems. (A) Serum C3 level was significant lower in cirrhosis with C-P C compared with C-P A/B ($p<0.0001$); (B) C3 was observed to be significantly negatively correlated with MELD score; (C) C3 was significance negative correlation with AST to platelet ratio; (D) In spite of C4 level was significance difference between three C-P score groups ($p<0.0001$), There was no significant differences between B and C ($p=0.293$); (E) C4 was observed to be significantly negatively

correlated with MELD score; (F) C4 was significance negative correlation with AST to platelet ratio. C-P score, Child-Pugh score; MELD score, End-stage Liver Disease score.

Figure 5

Complement 3 and 4 levels in decompensated HBV-cirrhosis with infection or died. (A) C3 level was significantly lower in decompensated cirrhosis with infection compared to without ($p < 0.0001$); (B) C4 level was significantly lower in decompensated cirrhosis with infection compared to without ($p = 0.002$); (C) The receiver operating characteristic (ROC) curve was applied to predict the optimal cut-point of C3 and C4 levels on decompensated HBV-cirrhosis with bacterial infection, the optimal cut-point of C3 level for predicting infection was 0.425 g/L (sensitivity: 72.7%, specificity: 84.7%), the C4 level was 0.095 g/L (sensitivity: 66.7%, specificity: 63.1%), the area under curve of C3 (AUC=0.817, 95%CI: 0.735-0.9, $p < 0.0001$) was markedly higher than C4 (AUC=0.671, 95%CI: 0.564-0.779, $p = 0.002$); (D) C3 level was significantly lower in died decompensated cirrhosis compared to survived ($p < 0.0001$); (E) C4 level was significantly lower in died decompensated cirrhosis compared to survived ($p = 0.014$); (F) According to ROC curve, the optimal cut-point of C3 level for predicting death was 0.415 g/L (sensitivity: 80%, specificity: 82.4%), the C4 level was 0.065 g/L (sensitivity: 45%, specificity: 84.1%), the area under curve of C3 (AUC=0.829, 95%CI: 0.725-0.933, $p < 0.0001$) was markedly higher than C4 (AUC=0.669, 95%CI: 0.533-0.806, $p = 0.013$); (G) Kaplan-Meier survival analysis revealed that significantly worse survival rate in low C3 level grade compared with high group; (H) Kaplan-Meier survival analysis revealed that high C4 level group were better in survival rate of decompensated cirrhosis than low group. IF, infection; P, present; N, no.