

Complement factor 3 among children with hepatitis A: Assessment of bilirubin levels

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Abstract

Objective: To evaluate the complement factor 3 levels in children with hepatitis A.

Methods: This observational study was conducted at the Infectious Diseases Hospital of Hotan District, China, from September 2014 to January 2015, and comprised children with hepatitis A and controls. The patients were divided into two groups. The ones with total bilirubin less than or equal to 2mg/dl comprised group A, while the ones whose total bilirubin was more than 2mg/dl was named group B. Besides, we enrolled age- and gender-matched healthy children as controls. SPSS 13 was used for data analysis.

Results: Of the 100 participants, 41(41%) were in group A, 29(29%) in group B and 30(30%) were controls. The serum level of alanine aminotransferase, aspartate aminotransferase, total bile acid, the incidence of ascites and the incidence of hepatic encephalopathy were significantly increased in patients of group B when compared to group A ($p=0.046$, $p=0.009$, $p<0.0001$, $p=0.018$ and $p=0.026$). The levels of prothrombin time activity, total protein and albumin were higher in group A ($p<0.0001$, $p<0.0001$, and $p<0.0001$). Total hepatitis A patients had significantly lower serum complement factor 3 levels compared to normal controls ($p=0.018$). Group B had significantly lower serum complement factor 3 levels compared to normal controls ($p<0.0001$) and group A ($p<0.0001$). In total patients, complement factor 3 levels were negatively correlated with total bilirubin and alanine aminotransferase ($p=0.029$), while complement factor 3 levels were positively correlated with prothrombin time activity ($p=0.001$).

Conclusion: Complement factor 3 values were found to be decreased in children hospitalised with hyperbilirubinaemia hepatitis A.

Keywords: Viral infection, Child, Inflammation, Cytokines. (JPMA 68: 21; 2018)

Introduction

Hepatitis A virus (HAV) infection is the most common cause of acute viral hepatitis, with approximately 1.5 million cases reported globally each year.¹ The primary modes of HAV transmission are contact with an infected person and ingestion of contaminated water or food.² Although HAV infection mainly causes self-limited symptoms, acute liver failure following HAV infection is occasionally observed. The mechanism of acute liver failure caused by HAV infection is not yet fully understood.³

Changes in the bilirubin have been associated with liver injury during hepatic infection. Bilirubin, a potent endogenous antioxidant, has been shown to be an immunomodulator. In addition, bilirubin is able to decrease interleukin 2 (IL-2) production in human lymphocytes.⁴ The complement can be activated via the classical, lectin, or alternative pathways, resulting in C3

(the third component of the complement) activation and leading to the generation of the membrane attack complex (C5b-9). Complement activation has also been reported to activate multiple immune cells and play an important role in host defence and wound healing by increasing secretion of inflammatory cytokines.⁵ However, it is not clear if the complement, which is an important bridge between the innate and adaptive immune systems, plays a role in the pathogenesis of hepatitis A. Therefore, we hypothesised that the interplay between different levels of bilirubin and C3 may modulate the immune response to HAV and influence the severity of disease.

The current study was planned to investigate C3 in patients with hepatitis A at different levels of bilirubin and compared results with healthy control subjects.

Patients and Methods

This observational study was conducted at the Infectious Diseases Hospital of Hotan District, China, from September 2014 to January 2015, and comprised children aged 1 to 15 years who were hospitalised with hepatitis A. The protocol was approved by the Beijing

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Youan Hospital's ethics committee. Informed consent was obtained from the next-of-kin of the subjects.

Patients meeting the clinical case definition of hepatitis A of the Centres for Disease Control and Prevention (CDC) were included. The CDC defines hepatitis A as "an acute illness with discrete onset of symptoms and either jaundice or elevated serum aminotransferase levels and a positive immunoglobulin M antibody to HAV (anti-HAV) serologic result".⁶ Patients with the following conditions were excluded: presence of other liver diseases including autoimmune liver diseases, Wilson's disease, etc; co-infection with hepatitis B, C, D, or E or human immunodeficiency virus (HIV); history of renal, cardiovascular, pulmonary, endocrine or rheumatic diseases. Patients with any other infectious diseases or chronic diseases, including haematologic and hepatic diseases, were also excluded. Moreover, 10 patients refused to participate in the study and 4 patients were excluded from the final analysis for the following reasons: 1 patient had hepatitis B virus (HBV), 1 patient had hepatitis C virus (HCV) and 2 patients had other hepatic diseases (Figure-1). Each patient was treated with the same comprehensive supportive treatment, including conventional liver protecting treatment. The selected patients were divided into two groups. Total bilirubin (TBIL) was less than or equal to 2 mg/dl in group A and greater than 2 mg/dl in group B. We also enrolled healthy controls. Peripheral blood was collected and the serum was separated and analysed immediately.

Serum concentrations of C3 and C4 were analysed using kits from Siemens Medical Solutions Diagnostics (Munich, Germany) according to the manufacturer's protocol. All values were compared to the normal ranges which were reported as 0.9-1.8g/L for C3, 0.1-0.4g/L for C4.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), TBIL, albumin (ALB), total bile acid (TBA), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) were quantitated using an autoanalyser (SYSMEX, Tokyo, Japan). Prothrombin time (PT) and prothrombin time activity (PTA) were measured using an automatic haemostasis/thrombosis analyser (PRECIL, Beijing, China).

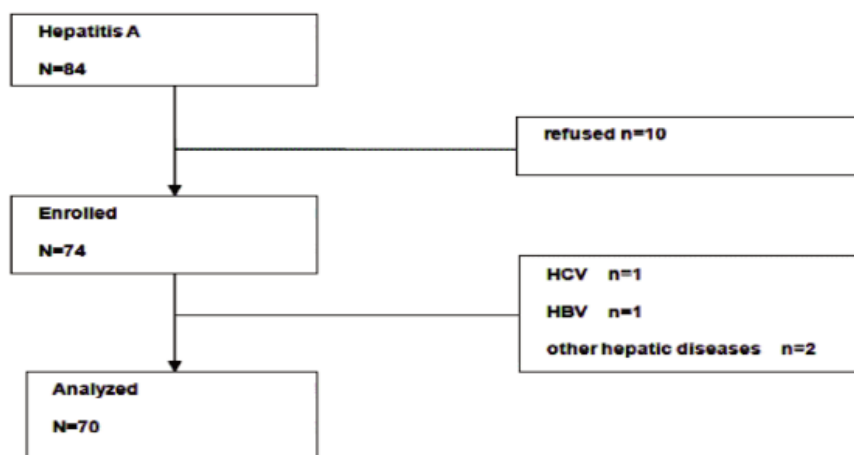
Statistical analysis was performed using SPSS 13. All the parametric data was expressed as mean \pm standard

deviation (SD) and differences between the two groups were assessed by Student's t-test; non-parametric data was expressed as median (range) and differences between the two groups were assessed by a Wilcoxon rank-sum test. For the data expressed as percentages, the differences between the two groups were assessed by a chi-square test. Correlation analysis was evaluated by the Spearman's rank correlation test. $P < 0.05$ was considered statistically significant.

Results

Of the 100 participants, 41(41%) were in group A 29(29%) in group B and 30(30%) were controls. No significant differences existed among the groups for the age and gender ratio ($p=0.54$ and $p=0.898$). The serum levels of ALT, AST, TBA, the incidence of ascites (1(2.4%) in group A and 6(20.7%) in group B) and the incidence of hepatic encephalopathy (HE) (none in group A and 4(13.8%) in group B) were significantly increased in patients with group B when compared to the ones in group A ($p=0.046$, $p=0.009$, $p < 0.0001$, $p=0.018$ and $p=0.026$). PTA, total protein (TP) and ALB were higher in group A ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$) (Table-1).

The patients had significantly lower serum C3 levels compared to controls ($p=0.018$). No significant differences existed in C3 levels between group A and controls ($p=0.513$). Group B had significantly lower serum C3 levels compared to controls ($p < 0.0001$) and group A ($p < 0.0001$). Serum C4 levels were not significantly different in controls compared to overall patients, group A and group B ($p=0.334$, $p=0.234$ and $p=0.678$, respectively). No significant difference existed in C4 levels between group A and group B ($p=0.461$).



HCV: Hepatitis C virus. HBV: Hepatitis B virus

Figure-1: Selection and deposition of patients.

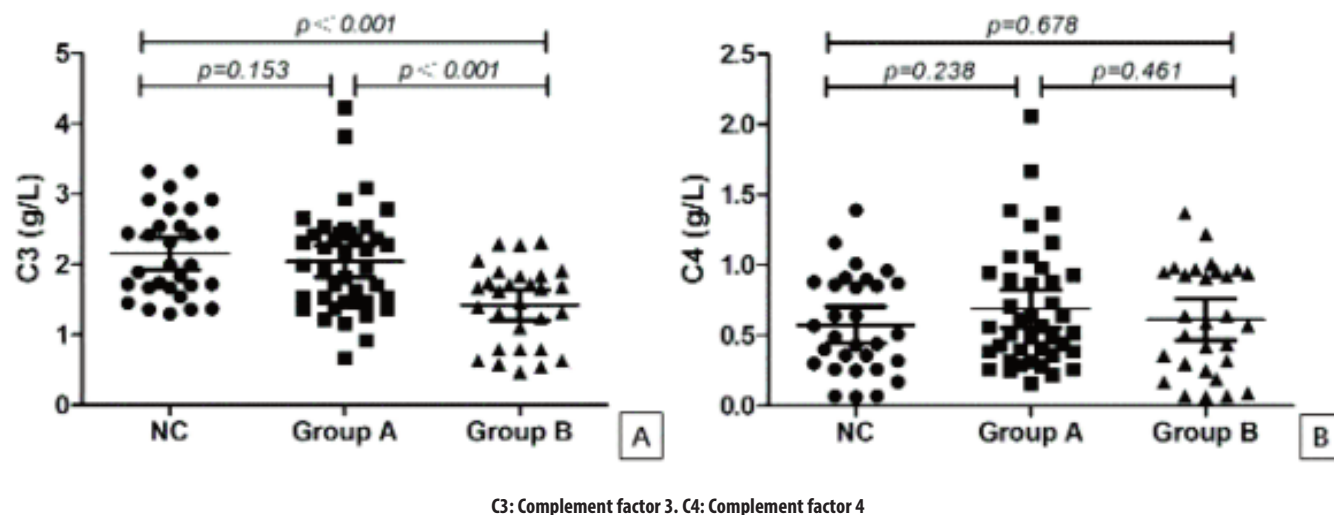


Figure-2: Serum C3 and C4 levels in different groups.

Table-1: Clinical characteristics of the populations enrolled in the study.

Characteristics	Total	Group A	Group B
Number of patients	70	41	29
Age (y)	3(1,15)	3(1,12)	3(1,15)
Male, n (%)	42(60%)	26(63.4%)	16(55.2%)
ALT (IU/L)	128(14,1780)	83(28,1510)	190(14,1780)
AST (IU/L)	75(10,1026)	56(26,666)	117(10,1026)
Tbil	18.1(2.6,373.8)	11.3(2.6,19.8)	51.8(34.3,373.8)
PTA (%)	76.8±19.8	84.2±13.2	66.2±22.8
TP (g/L)	69.8±6.6	71.9±5.1	66.9±7.5
Albumin (g/L)	40±4.2	41.9±2.8	37.3±4.5
ALP (IU/L)	252(76,1052)	252(85,608)	234(76,1052)
GGT (IU/L)	75(11,448)	69(19,320)	123(11,448)
TBA (?mol/L)	N.D.	8(0.5,32)	17(2,223)
HE, n (%)	0	0	4 (13.8%)
Ascites, n (%)	0	1 (2.4%)	6 (20.7%)
liver failure, n (%)	0	0	4 (13.8%)

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

TBIL: Total bilirubin

PTA: Prothrombin time activity

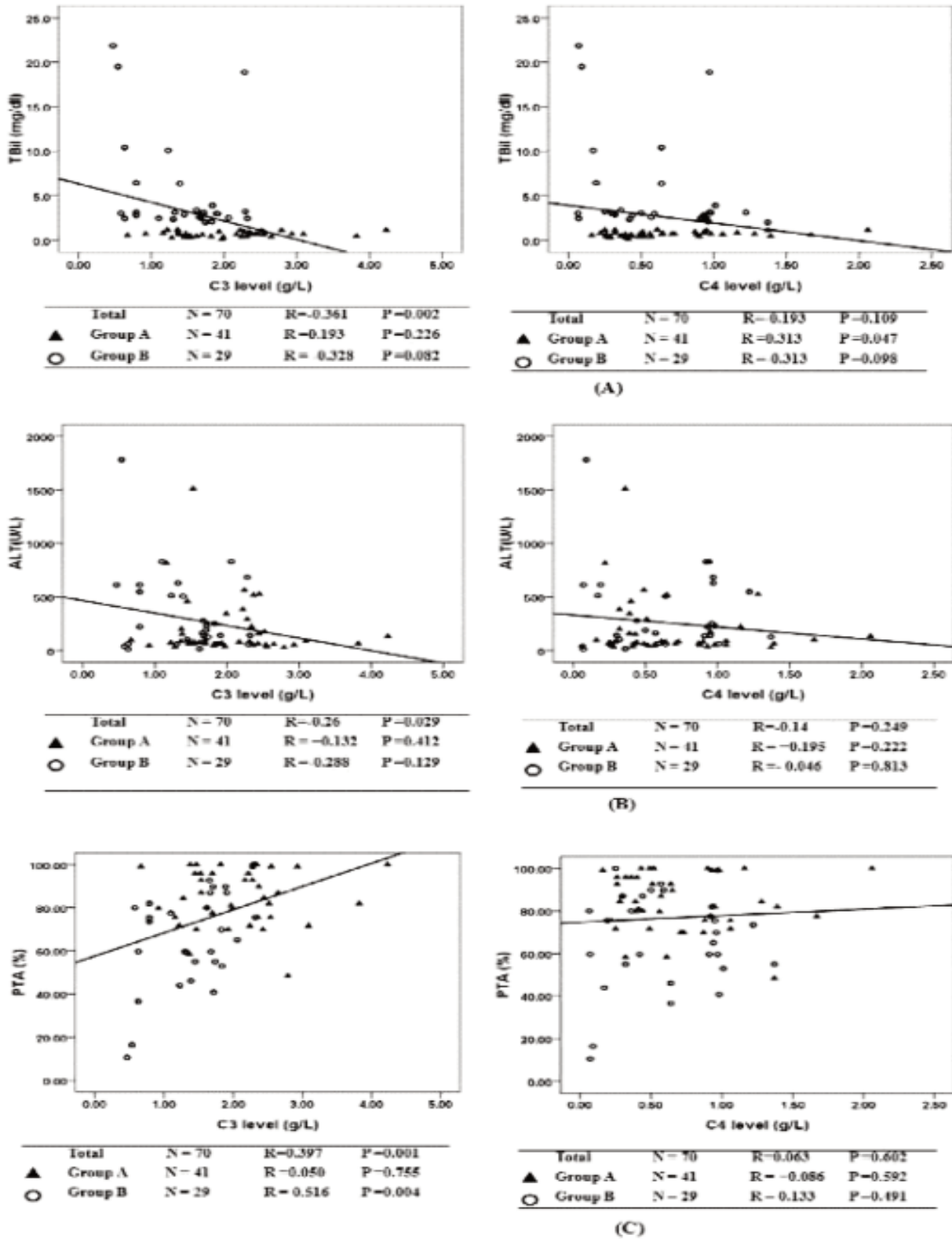
TP: Total protein

Data with normal distribution are expressed as mean ± standard deviation. And categorical data were expressed as median (range).

(Figure-2A and 2B) (Table-2).

We analysed the correlation between serum C3, C4 levels and parameters for disease severity in hepatitis A patients. In total patients, C3 levels were negatively correlated with TBIL and ALT ($r = -0.361$, $p=0.002$; $r = -0.26$, $p=0.029$), while C3 levels were positively correlated with PTA ($r=0.397$, $p=0.001$). However, C4 levels were not significantly associated with TBIL, ALT and PTA ($r = -0.193$, $p=0.109$; $r = -0.14$, $p=0.249$; $r = 0.063$, $p=0.602$). In group

A, C3 levels were not significantly associated with TBIL, ALT and PTA ($r=0.193$, $p=0.226$; $r = -0.132$, $p=0.412$; $r=0.05$, $p=0.755$). C4 levels were not significantly associated with ALT and PTA ($r = -0.195$, $p=0.222$; $r = -0.086$, $p=0.592$), while C4 levels were positively correlated with TBIL ($r=0.313$, $p=0.047$). In group B, C3 levels were not significantly associated with TBIL and ALT ($r = -0.328$, $p=0.082$; $r = -0.288$, $p=0.129$), while C3 levels were positively correlated with PTA ($r=0.519$, $p=0.004$).



TBil: Total bilirubin. PTA: Prothrombin time activity. C3: Complement factor 3. C4: Complement factor 4.

Figure-3: C3 and C4 levels indicated liver injury in hepatitis A patients.

Table-2: Comparison of complement factors in different groups.

Group	C3	C4
Healthy controls	2.15±0.62	0.57±0.35
Total	1.79±0.73	0.66±0.41
Group A	2.04±0.73	0.69±0.43
Group B	1.42±0.57	0.61±0.38
Healthy controls vs A P value	0.513	0.238
Healthy controls vs B P value	<0.0001	0.678
Healthy controls vs Total P value	0.018	0.334
A vs. B P value	<0.0001	0.461

Data with normal distribution are expressed as mean±standard deviation. T-test, $p < 0.05$.

However, C4 levels were not significantly associated with TBIL, ALT and PTA ($r = -0.313$, $p = 0.098$; $r = -0.046$, $p = 0.813$; $r = 0.133$, $p = 0.491$) (Figure-3).

Discussion

To the best of our knowledge, this is the first study to evaluate C3 and C4 in different levels of bilirubin among children with hepatitis A. We demonstrated that clinical manifestation of hyperbilirubinaemia patients was more serious, C3 levels were significantly lower in hyperbilirubinaemia patients, and lower C3 levels were closely correlated with liver injury and impaired liver regeneration.

Elevated bilirubin is a common clinical manifestation of hepatitis A. A previous study showed that bilirubin can suppress inflammation and increase antioxidant enzyme generation in activated neonatal neutrophils by down-regulating the lipopolysaccharide-induced generation of IL-8.^{7,8} Conjugated bilirubin can induce IL-6 and tumour necrosis factor alpha (TNF- α) secretion in human peripheral blood lymphoid cells (PBLs) in a dose-dependent manner.⁹ Arai T. et al. showed that the bilirubin concentration in the buffer solution was decreased by the addition of hydrogen peroxide, especially in the presence of peroxidase or ferrous iron. dichlorofluorescein diacetate oxidation by reactive oxygen species (ROS) or activated neutrophils was inhibited by bilirubin in a dose-dependent manner. The bactericidal activity of ROS or of isolated neutrophils was significantly attenuated by bilirubin.¹⁰ Moreover, the extraordinary antioxidant property of bilirubin may contribute to its immunomodulatory action by regulating cytokine production during HAV infection. During HAV infection, high concentrations of bilirubin may affect cytokine secretion by immune cells, including neutrophils.⁹ In our study we found that in patients with hepatitis A, ALT, AST, TBA, the incidence of ascites and the incidence of hepatic encephalopathy values of hyperbilirubinaemia patients were significantly higher than those who were not

hyperbilirubinaemia patients. The conclusion of the PTA values was the opposite. This finding in our study may reflect the inflammatory process in hepatitis A.

The complement system is the major effector of the humoral arm of the immune system. Its third component (C3) is the most abundant complement protein in the circulation and plays a pivotal role in the complement cascade. Accumulating data have indicated a pathogenic role of C3 in a variety of liver diseases.¹¹⁻¹³ However, the role of the complement in HAV patients is poorly understood. The liver is composed of hepatocytes, Kupffer cells, stellate cells and sinusoidal endothelial cells. In contrast to hepatocytes, normal Kupffer cells express complement receptors for both C3 at high levels.^{14,15} Several complement receptors have been detected in liver cells and contribute to a variety of functions in the liver. Complement has also been involved in liver regeneration after partial hepatectomy or after toxic injury.¹⁶⁻¹⁸ By using a murine model of partial hepatectomy, Strey et al. demonstrated that the anaphylatoxins C3a are essential for liver regeneration.¹⁷ C3 deficiency results in diminished liver regeneration, accompanied by transient or fatal liver failure after partial hepatectomy.¹⁷ Müller et al. demonstrated that production of TNF by peripheral blood monocytes was reduced in patients with acute hepatitis B infection but not in those with acute hepatitis A.¹⁹ On the other hand, Muto et al. reported normal levels of TNF- α in patients with acute hepatitis A or B without fulminant liver failure.²⁰ Müller et al. studied IL-6 and IL-1 levels in patients with hepatitis A in two different studies. He demonstrated normal levels of IL-6 and severely decreased during the first week and gradually increased IL-1 levels during the further course of hepatitis A.²¹ Fierro et al. reported an over-expression of TNF- α , IL-1 and IL-6 in children with HAV-induced intermediate liver damage while minor liver damage was characterised by increase of IL-8 and transforming growth factor beta, suggesting that an imbalance in the inflammatory cytokines occurs during the course of hepatitis A.²² In addition, expression of many complement components in the liver is significantly changed during acute phase response and induced by proinflammatory cytokines such as IL-6, IL-1, TNF- α and IFN- γ .²³⁻²⁵ The reason of these arguments may be related to choose different period and disease severity of hepatitis A patients. Our data suggests that C3 was closely correlated with liver injury in hyperbilirubinaemia patients. There are several lines of evidence to support this notion. C3 levels were negatively correlated with serum TBIL and ALT levels, but positively correlated with plasma PTA levels, which often serve as markers of liver injury.²⁶ These results suggest that complement was closely associated with hyperbilirubinaemia patients liver

damage, which may contribute to the pathogenesis of HAV.

The complement system consists of about 30 soluble and membrane bound proteins, and is activated by 3 distinct pathways either on pathogen surface or in plasma. Activation of these pathways depends on different molecules for their initiation. The classical pathway is triggered by antigen-bound antibody molecules and is initiated by the binding of a specific part of the antibody molecule (Fc) to the C1 component.²⁷ The alternative pathway is a humoral component of the immune system's natural defence against infections and is activated by cleavage of C3 and then C5. The mannose-binding lectin (MBL) pathway is initiated when the plasma MBL protein forms a complex with the MBL-associated proteases 1 and 2 (MASP1 and MASP2). MASP1 and MASP2 then bind to arrays of mannose groups on the surface of a bacterial cell.²⁸ C3 and C5 are the key element of the complement cascade activation system and markers. C4 is not activated by every distinct pathway. In our research, C4 levels were not significantly associated with TBIL, ALT and PTA. We need to conduct further research to find a reason.

The current study had two important limitations. Firstly, we did not analyse the complement levels in the microenvironment of liver tissues in HAV patients due to poor coagulation, which may be a contraindication for liver biopsy. Secondly, this study consisted of children with hepatitis A who were hospitalised; thus, further studies are needed for outpatients and the adult age group. Future studies to explore the mechanisms underlying the role of complement in HAV patients are urgently needed.

Conclusion

Clinical manifestation of hyperbilirubinaemia patients was more serious, C3 levels were significantly lower in hyperbilirubinaemia patients, and lower C3 levels were closely correlated with liver injury and impaired liver regeneration, suggesting that the complement may be implicated in the pathogenesis of HAV. However, prospective studies with larger number of patients are needed to assess the mechanism of decreased C3 values in hepatitis A.

Disclaimer: None.

Conflict of Interest: None.

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