Serum Levels of Complement C1Q, C3 and C4 in Patients at Different Stages of Chronic Hepatitis C Viral Infection

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Abstract: Hepatitis C Virus (HCV) is a leading cause of chronic liver disease worldwide. The involvement of complement in the course of HCV infection has been poorly documented. The aim of this study was to analyze the association between C1Q, C3 and C4 and the clinical stage of HCV infection. Eighty-two HCV-infected patients and 20 controls were enrolled in this work. Serum C1Q Circulating Immune Complexes (CIC) were measured using ELISA while radial immunodiffusion was used to measure serum C3 and C4 complement. HCV-infected patients were classified into 20 patients with fatty liver, 60 patients with cirrhotic liver and 22 patients with HCV-related Hepatocellular Carcinoma (HCC). Twenty healthy individuals served as normal controls. Positive correlation was recorded between the age of the patients and the progress of the disease. Progress of HCV-infection was coinciding with elevation in C1Q level, decrease in C3 and C4 level with maximum reduction in patients with severe cirrhosis then re-increase in HCC patients. The magnitude of liver cirrhosis was positively correlated with C1Q and negatively correlated with both C3 and C4 levels. HCC patients have a different production level of complement compared to patients with cirrhotic liver. In conclusion, elevation of C1Q level could be used as an indication for the progress of cirrhosis in HCV-infected patients while its reduction could be employed as a potential predictive value for detection of HCC in patients with liver cirrhosis.

Key words: HCV · Liver · Cirrhosis · HCC · Complement

INTRODUCTION

Infection with hepatitis C Virus (HCV) remains a severe life-threakning medical and public health problem worldwide. The estimated global prevalence of HCV infection is 2.2%, corresponding to about 130 000 000 HCV-positive persons worldwide [1]. The highest prevalence has been reported from Egypt [2-4] where the main (90%) HCV genotype is type 4 [5-7]. An estimated 27% of cirrhosis and 25% of HCC worldwide occur in HCV-infected people [2].

The most remarkable feature of HCV is its ability to efficiently establish persistent infection by evading host immune surveillance [8]. Interactions between HCV and the host immune system play an important role in HCV persistence and disease pathogenesis [9]. Complement represents a significant non-specific host defense system involved in the protection of the host from viral infection [10]. To escape this protection, viruses are able to express host-homogenous proteins, or to borrow cell membrane proteins from the host with complement regulatory activity, protecting viral particles from neutralization by complement [11]. The involvement of complement in the course of HCV infection has been poorly documented. A few studies show an association between HCV infection and a cold-dependent activation of the classical complement pathway [12, 13] or hypocomplementemia associated with cryoblobulinemia [14]. Specific C4 monitoring appeared to be a valuable tool for the follow up of chronic HCV treatment [9]. Thus, the aim of this study was to evaluate the potential impact of complement C1Q, C3 and C4 during the course of HCV infection in order to get more description and understanding to the role of complement system in the disease progression.

MATERIALS AND METHODS

The present study was performed on 122 subjects (82 HCV-infected patients and 20 negative controls)

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attending the out patient clinic of National Liver Institute, Minofiya University, Egypt. All investigations were done in accordance with the Minofiya University, health and human Ethical Clearance Committee guidelines for clinical researches. Local ethics committee approved the study protocol. All patients and controls agreed to be enrolled in this study.

All participants were subjected to thorough history taking and clinical examination, abdominal ultrasonography and liver biopsy where indicated, HCV antibody assay by 3rd generation Enzyme Linked Immunosorbent Assay (ELISA) (Murex Biotech) confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) (Promega Co. MA, USA) and Hepatitis B surface antigens (HBsAg) (Sorin Biomedica Co., Italy) using commercially available kits. All patient groups were HCV antibody and HCV-RNA positive. They were all negative for HBsAg (by ELISA) and schistosome infection (by parasitological examinations). None of the patients had received antiviral therapy or had a history of habitual alcohol consumption. Urine [15] and stool [16] examinations as well as sigmodiscopy [17] were carried out. Exclusion criteria included co-infection with HBV and/or schistosome infection.

Sera were collected form all patients and controls. They were separated by centrifugation at 1500 rpm for 15 min at 4°C, aliquoted and kept at -70°C until used.

Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) (BioMerieux, France) direct and indirect bilirubin (Roche Diagnostics, LTD, USA) and albumin (Human Gesellschaft Fur Biochemica Und Diagnostica Mbh, Wiesbaden, Germany) were measured according the kit's instruction manual.

Serum C1Q Circulating Immune Complexes (CIC) were measured using ELISA (DRG International Inc., USA). The ELISA-reader controlling software (Softmax) was used to processes the digital data of raw absorbance value into a 4-parameter standard curve from which the content of unknown samples can be derived directly. Serum C3 and C4 complement concentrations were estimated by radial immunodiffusion method using commercially available kits (BINDARID™, The Binding Site Ltd, Birmingham, UK). By measuring the ring diameters produced by number of samples of known concentrations (calibrators), a calibration curve was constructed. The concentration of the complement in tested samples was determined by measuring the ring diameter produced by that sample and reading off the calibration curve.

Data were expressed as mean ± SEM. Data were analyzed using the statistical software package for social science (SPSS). Comparisons among different groups of patients were performed by one-way Analysis of Variance (ANOVA). Tukey was used as post-hoc test [18].

RESULTS

HCV-infected patients were classified using necroinflammatory grading system [19] into the following groups: 20 patients with Fatty Liver (FL) (no cirrhosis), 60 patients with cirrhotic liver (20 patients mild (MI), 20 patients moderate (MO) and 20 patients severe (SE) cirrhosis) and 22 patients with HCV-related HCC. Twenty healthy individuals with no history of previous liver affection, with normal liver function tests and negative serology of HCV antibody served as normal controls. As shown in Table 1, most of patients are males. Patients with more severe cirrhosis are generally older. Positive correlation was recorded between the age of the patients and the progress of the disease (r=0.544, $P<0.001$).

Gradual increase was observed in bilirubin, ALT, AST and ALP levels in different stages of cirrhosis. On the other hand, progression of HCV infection was accompanied by reduction in albumin level (Table 2). A positive association was recorded between the level of cirrhosis and AST (r=0.360, $P<0.001$). An inverse correlation was found between the level of cirrhosis and albumin production (r=-0.762, $P<0.001$). HCC patients were characterized by increase in all biochemical markers of liver function (bilirubin, ALT, AST and ALP) and sharp reduction in albumin level.

Significant increase in C1Q was estimated in patients with HCV infection ($P<0.001$) compared with normal controls (Fig. 1). Comparing different grades of HCV-infection revealed that patients with severe cirrhosis produced significantly more C1Q than did patients with fatty liver, mild cirrhosis and HCC ($P<0.01$). A reduction in C1Q level was found in HCC patients. A positive association (r=0.528, $P<0.001$) between the magnitude of liver cirrhosis and the production of C1Q was recorded.

Concerning C3, general reduction in C3 level was reported in HCV-infected patients with cirrhotic liver followed by sharp increase in HCC patients (Fig. 2). In relation to normal controls, although there is a reduction in C3 level, the significant decrease was recorded only in patients with severe cirrhosis ($P<0.001$). Comparing patients at different stages of infection showed a significant increase in HCC in comparison with patients with fatty liver ($P<0.01$) mild ($P<0.01$), moderate ($P<0.001$)

Table 1: Demographic data of different groups of patients with chronic HCV-infection and normal controls

	Group					
Characteristics	Control	FL	MI	MO	SE	HCC
Number	20	20	20	20	20	22
Age:						
Mean±SD	33.9±4.3	44.3±6.4	49.9±9.6	57.0±7.1	56.6±6.5	55.3±4.3
Range	27-42	32-59	39-75	45-68	43-69	43-68
Sex:						
M (♂)	19 (95%)	14 (70%)	16 (80%)	15 (75%)	16 (80%)	20 (91%)
F (♀)	1 (5%)	6 (30%)	4 (20%)	5 (25%)	4 (20%)	2 (9%)
Occupation:						
Employee	16 (80%)	12 (60%)	14 (70%)	10 (50%)	9 (45%)	12 (54%)
Farmer	4 (20%)	6 (30%)	5 (25%)	7 (35%)	10 (50%)	8 (37%)
House wife	---------	2 (10%)	1 (5%)	3 (5%)	1 (5%)	2 (9%)
Liver:						
Normal size	20 (100%)	19 (95%)	11 (55%)	5 (25%)	6 (30%)	9 (41%)
Enlarged	-	-	9 (45%)	11 (55%)	5 (25%)	7 (32%)
Small	-	-	-	4 (20%)	9 (45%)	6 (27%)
Spleen:						
Normal size	20 (100%)	20 (100%)	20 (100%)	13 (65%)	9 (45%)	10 (45%)
Enlarged	-	-	-	6 (30%)	11 (55%)	10 (45%)
Spleenectomy	-	-	-	1 (5%)	-	2 (9%)
HCV:						
Ab	-	+	+	+	+	+
PCR	-	+	+	+	+	+
HBVsAg	-	-	-	-	-	-
Schistosome infection	-	-	-	-	-	-

Table 2: Data of liver function tests of different patient groups with chronic HCV-infection, HCC and normal controls ($^aP<0.05$ and $^bP<0.01$)

	Group						
Parameter	Controls	FL	MI	MO	SE	HCC	Sig.
D. bilirubin	0.20±0.02	0.20±0.02	0.35±0.04	1.01±0.14	0.57±0.06	0.98±0.27	b
T. bilirubin	0.83±0.03	0.88±0.03	1.06±0.08	3.29±0.72	1.77±0.16	3.30±0.85	b
ALT	15.95±1.12	26.45±2.25	28.00±3.58	28.00±3.58	31.90±4.07	28.72±5.23	a
AST	22.20±1.36	36.95±2.28	41.05±2.96	63.75±10.97	68.15±10.16	91.59±21.06	b
ALP	62.25±2.95	66.55±4.07	79.15±7.03	77.00±9.89	85.45±6.63	106.72±15.71	b
Albumin	4.14±0.05	3.91±0.05	2.41±0.14	1.98±0.06	2.08±0.06	2.00±0.07	b

and severe cirrhosis ($P<0.001$). A negative correlation between the progress of cirrhosis and the level of C3 was depicted (r = -0.328, $P<0.001$).

As illustrated in Fig. 3, transient increase in C4 levels were observed in patients with mild or moderate cirrhosis followed by a sharp reduction in patients with severe cirrhosis. In relation to uninfected controls, significant elevation was recorded in patients with mild cirrhosis and in HCC patients ($P<0.001$). Comparing patients at various clinical forms of HCV infection approved a significant decrease in patients with severe cirrhosis ($P<0.01$) and significant increase in HCC ($P<0.01$) patients. An inverse correlation (r = -0.299, $P<0.001$) between the progression of cirrhotic liver and the C4 level was found.

Thus, progression of cirrhotic liver was coinciding with elevation in C1Q level, reduction in C3 and gradual increase in C4 level followed by sharp reduction in
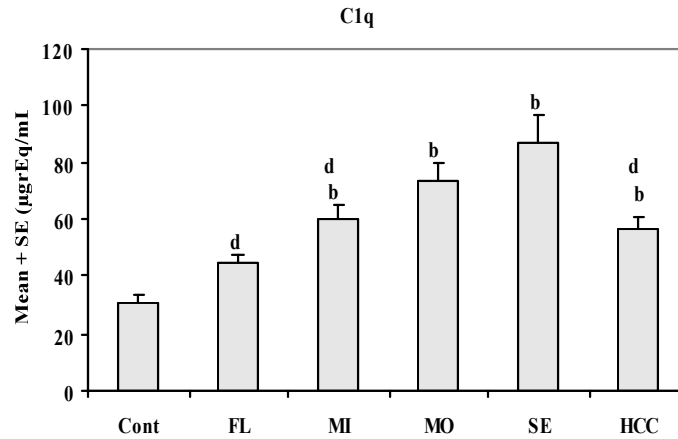
C1q

Fig. 1: C1q level in patients at different stages of HCV-infection. Results are expressed as mean±SEM.[b] *P*<0.01 denotes group statistically significant from controls. [d]*P*<0.01 denotes groups statistically significant from patients with severe cirrhosis

C3
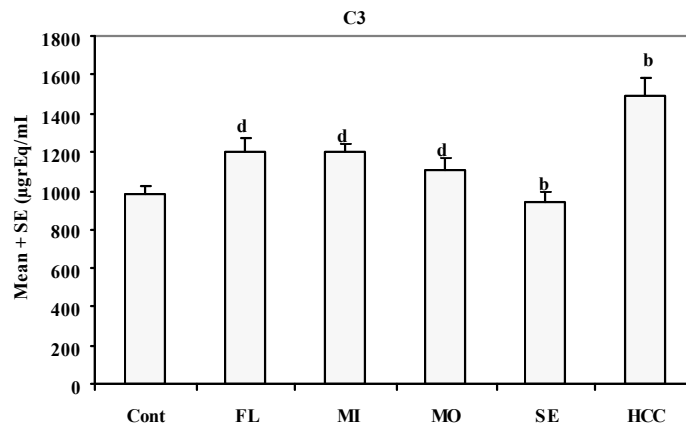
Fig. 2: C3 level in patients at different stages of HCV-infection. Results are expressed as mean±SEM. [b]*P*<0.01 denotes group statistically significant from controls. [d]*P*<0.01 denotes groups statistically significant from HCC patients

C4

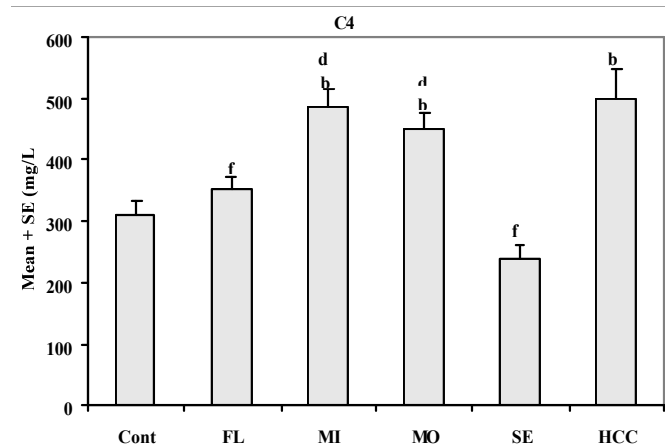
Fig. 3: C4 level in patients at different stages of HCV-infection. Results are expressed as mean±SEM. [b]*P*<0.01 denotes group statistically significant from controls. [d]*P*<0.01 denotes groups statistically significant from patients with severe cirrhosis. [f]*P*<0.01 denotes groups statistically significant from HCC patients

patients with severe cirrhosis then re-increase in HCC patients. The level of liver cirrhosis was positively correlated with C1Q level and negatively correlated with both C3 and C4 levels. HCC patients have a different production level of complement compared to patients with cirrhotic liver.

DISCUSSION

The complement system is one of the most important weapons of innate immunity and is involved in all infectious processes. It is not only a mechanism for direct protection against an invading pathogen but it also interacts with the adaptive immunity to optimize the pathogen-specific humoral and cellular defense cascade in the body [20]. Only scarce data on the levels of complements in chronic liver hepatitis patients [21] are available. Most findings concern an association between HCV infection and a cold-dependent activation of the classical pathway [12, 13] or lymphocomplementemia associated with cryoglobulinemia [14]. Thus, the aim of the present work was to study the changes in C1Q, C3 and C4 in chronic HCV-infected patients and their possible correlation with the progress of the disease which may yield valuable insights into the disease mechanism.

One hundred and two patients suffering from chronic liver hepatitis were enrolled in this study. They were classified according to the necroinflammatory grading system [19]. As shown in our results, patients with cirrhotic liver are generally older. The incidence of liver cirrhosis was significantly increased by aging. The time taken to develop cirrhosis was shorter in older age patients (14±7 years) for those infected before age of 40 years as compared to 8±5 years in patients infected after 40 years [22]. The same results were confirmed by [23] who reported that the prevalence of HCV related infection is age dependent. The highest HCV prevalence in the world occurs in Egypt, where the prevalence of infection increases steadily with age and high rates of infection are observed among persons in all age groups [4, 24, 25]. On the other hand, this study approved that the development of HCC is age irrelevant. The age is an independent risk factor for HCC in patients with liver cirrhosis [26].

In our study, significant increase in C1Q was estimated in patients with HCV infection compared with normal controls. The maximum production of C1Q was observed in patients with severe cirrhosis. In accordance with our results, high levels of serum C1Q were previously demonstrated in the advanced liver diseases [27, 28].

Among chronic cases, the C1Q level was the highest in liver cirrhosis sera and its serum levels seemed to increase with the progression of liver damages [28]. The mechanism of high serum C1Q concentrations in liver cirrhosis is not completely known. However, it could be speculated that the low complement activity in liver cirrhosis sera might not be brought on by the activation of classical complement pathway. It seems plausible to consider such a phenomenon as C1Q-bypath activation to be employed in cases with chronic liver diseases [29, 30]. A marked increase of fractional catabolic rates of C1Q in chronic liver disease was previously reported [31]. Taking in consideration that C1Q synthesis takes place outside the liver as hepatocytes can only produce C1r and C1s but not C1Q, so it could be affected by the reduction in synthetic ability of the liver cells in response to HCV-infection [32, 33]. Moreover, many cell types including epithelial cells, fibroblasts and cells of the monocyte/macrophage lineage can produce C1Q [34]. In HCV infection, leukocytes infiltration and increase in Kupffer cell number could be the source of additional C1Q.

A negative association between the magnitude of C1Q production and the level of albumin was estimated through this study. This negative correlation with the biochemical parameters of protein synthesis, such as albumin, emphasized that C1Q rises as liver function decreases. A negative correlation between C1Q immune complexes and C4 level and a positive correlation with the immunoglobulin levels were found in the majority of patients with various liver diseases, while prothrombin time and albumin levels were negatively correlated with C1Q immune complex levels only in patients with chronic active hepatitis [35].

In the present work, comparing different stages of cirrhosis (mild, moderate and severe) showed a general reduction in C3 and C4 levels in HCV-infected patients with cirrhotic liver reaching to the lowest level in patients with severe cirrhosis. Serum C3 and C4 concentrations were decreased in cirrhotic patients compared with normal individuals [36]. The complement component levels were observed to be reduced in viral hepatitis, where the reduction in C3 serum concentration was found to be statistically significant but reduction in C4 serum concentration was not [37]. Excessive activation of plasma C3 in the fluminant hepatitis group was detected [38]. The C3d/C3 ratio was proved to be directly related to the severity of clinical symptoms. Serum complement concentrations of C3 and C4 correlate negatively with the Child-Pugh score in patients with liver cirrhosis. This is

meant that there is a decrease in C3 and C4 with the progression of liver cirrhosis [39].

Reduction in C3 and C4 concentrations in these patients may reflect complement consumption or reduced production due to a decline in the number of functioning hepatocytes. This hypothesis is supported by the simultaneous decrease in the concentrations of C4 and albumin, which are produced in the liver. Since the liver is the major site of synthesis of most of the complement components, the low serum complement level has been proposed to be induced by the defective synthesis of the components [40-42]. These results indicate that patients with hepatic disease have severe complement depletion that is probably multifactorial in origin. This impairment in complement function may be returned to two mechanisms: a failure to synthesis a certain number of components and regulatory proteins of complement and an increased consumption due to activation of the complement system. The increase consumption theory was supported by several reports [43,44]. This acquired deficit in complement contributes to the increased risk of infection in patients with cirrhosis [45, 46]. An association has been suggested between increasing severity of liver disease and increasing degrees of complement activation as shown by the C3d:C3 ratio being highest in patients with severe liver disease and by the correlations of C3d:C3 values with other markers of liver damage such as albumin, ALP and AST [47, 48]. This decline in the complement function will contribute to the impaired antibacterial host defense of the patient with chronic hepatic disease [46].

Our HCC patients showed a significant increase in C3 and C4 as compared to patients with chronic liver cirrhosis only. A lower level of C3 and C4 in patients with liver cirrhosis than in HCC patients was observed. Circulating immune complex and complement were significantly higher in HCC patients was previously demonstrated [49]. This imply that the increase of complement with tumor growth [50]. In HCC patients, increase in C3 and C4 were found to be a good diagnostic tool for HCC screening in liver cirrhosis patients and for follow-up evaluation in post-therapy patients of liver cirrhosis with HCC. Complement C3a was found to be elevated in patients with chronic HCV-related HCC [51].

Taken together, our data provide a detailed analysis of the changes in liver function and expression of complement C1Q, C3 and C4 among individuals with different clinical stage of HCV-infection. The progress of cirrhosis in HCV-infection was coinciding with elevation in C1Q level and reduction in C3 and C4 level. It is obvious from the presented data that levels of C1Q, C3 and C4 in chronic HCV-related HCC patients have a different production level compared to patients with cirrhotic liver without HCC complications. This difference could be attributed to the changes in different immunological parameters associated with tumor development.

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