

Serum complement C4 in chronic hepatitis C: Correlation with histopathologic findings and disease activity

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Background/aims: Hepatitis C virus leads to chronic liver disease, cirrhosis and hepatocellular cancer. Viral markers and other laboratory tests used in the diagnosis and follow-up of chronic hepatitis C do not correlate well with disease activity and liver histopathology. Therefore, alternative tests that indicate disease activity and relate with liver biopsy findings are needed. We aimed to investigate the relationship between serum complement levels and biopsy findings in patients with chronic hepatitis C. **Methods:** One hundred cases (70 patients, 30 healthy controls) were included in the study. Patients were divided into two groups: chronic hepatitis C patients with high transaminase levels were evaluated as the first group and patients with normal transaminase levels as the second group. Patients with a high transaminase level were biopsied and activity scores were evaluated against complement C3c and C4 levels. In addition, demographic data and laboratory tests were evaluated. Patients with chronic hepatitis C without proteinuria, acute phase response, cirrhosis, or coinfection with another hepatitis virus were included in the prospective study. **Results:** Serum complement C3c ($p<0.01$) and C4 ($p<0.01$) levels were significantly lower in the first group than the second group. Serum complement C3c levels did not correlate with laboratory tests, hepatitis C virus-RNA levels, histological activity index, or fibrosis scores in patients with high transaminase levels, whereas complement C4 levels showed significant correlation with alanine aminotransferase ($r: -0.368, p: 0.001$) and histological activity index ($r: -0.639, p: 0.001$). We could not find any relation between serum complement C4 level and fibrosis. **Conclusions:** Serum complement C4 levels correlate with the histological activity index of the Knodell scoring system.

Key words: Complement C4, Knodell HAI scoring, hepatitis C

Serum kompleman C4, kronik hepatit C histopatolojik bulgular ve hastalık aktivitesi

Amaç: Hepatit C virüsü kronik karaciğer hastalığı, siroz ve hepatosellüler kansere neden olmaktadır. Kronik hepatit C'nin tanı ve takibinde kullanılan viral marker ve laboratuvar testleri hastalığın aktivitesi ve karaciğer histopatolojik bulgularıyla iyi korelasyon göstermemektedir. Buna bağlı olarak karaciğer biyopsi bulguları ve hastalık aktivitesi ile uyumluluk gösteren testlere gereksinim vardır. Bu çalışmada kronik hepatit C hastalarında, serum kompleman düzeyi ile biyopsi bulguları arasındaki ilişkiyi araştırmak istedik. **Yöntem:** Yüz olgu (70 hasta ve 30 sağlıklı kontrol) çalışmaya alındı. Hastalar birincisi, yüksek transaminazlı kronik hepatit C hastaları ve ikincisi, normal transaminazlı kronik hepatit C hastaları olmak üzere ikiye ayrıldı. Yüksek transaminazlı hastalara biyopsi uygulandı ve aktivite skorları ile C3c ve C4 arasındaki ilişki değerlendirildi. Ayrıca demografik veriler ve laboratuvar testleri değerlendirildi. Bu çalışmaya proteinüri, akut faz yanıtı, siroz, diğer hepatit virüsleri ile birlikteliği olmayan kronik hepatit C hastaları alındı. **Bulgular:** Serum kompleman C3c ($p<0,01$) ve C4 ($p<0,01$) düzeyleri birinci grupta ikinci gruba göre anlamlı olarak daha düşüktü. Yüksek transaminazlı hastalarda serum kompleman C3c düzeyi laboratuvar testleri, hepatit C virüsü-RNA düzeyleri, histolojik aktivite indeksi ve fibrosis skoru ile korelasyon bulunmadı. Kompleman C4 düzeyleri ile alanin aminotransferaz ($r:-0,368$ ve $p:0,001$) ve histolojik aktivite indeksi ($r:-0,639$ ve $p:0,001$) ile anlamlı korelasyon vardı. Serum kompleman C4 ile fibrosis arasında anlamlı bir ilişki saptanmadı. **Sonuç:** Serum kompleman C4 düzeyi Knodell skorlama sistemindeki histolojik aktivite indeksi ile korelasyon gösterir.

Anahtar kelimeler: Kompleman C4, Knodell hai skorlaması, hepatit C

INTRODUCTION

Hepatitis C virus (HCV) infection is a frequent cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. It is pandemic around the globe and is estimated to afflict 150 to 200 million people worldwide. It results in a chronic disease in 85% of the cases (1,2).

The pathogenesis of HCV-induced hepatic injury remains unclear and could be attributed to either direct cytopathic damage by HCV- or immune-mediated hepatic injury, especially via cellular immunity (3-5).

Virological diagnosis and monitoring of chronic hepatitis C (CHC) infection is based on two categories of laboratory tests, namely serologic assays detecting specific antibodies to HCV (anti-HCV) and assays that can detect, quantify or characterize the components of HCV viral particles, such as HCV-RNA. These tests play a key role in the diagnosis of infection, therapeutic decision-making and assessment of virological response to therapy (6). The exact definition of the normal levels of serum alanine aminotransferase (ALT) activity is crucial for screening and follow-up studies in hepatitis C infection (7,8). Individually, none of these tests has sufficient diagnostic and prognostic utility. Anti-HCV immunoassay has a high false-positivity rate and may be falsely negative in spite of active viral replication in the first eight weeks after contracting the virus, in patients with immunosuppression, undergoing hemodialysis or with human immunodeficiency virus (HIV) infection (8-11). HCV-RNA can be detected in biopsy samples of patients with chronic liver disease of unknown etiology, who are then diagnosed with occult CHC (12). Serum ALT level is an essential but nonspecific test for monitoring the infection and assessing the effectiveness of treatment along with the molecular tests (13). However, ALT level may remain normal or fluctuate in patients with HCV infection, and observation of normal levels is not sufficient to rule out the presence of active infection, progressive liver disease or cirrhosis (1). Severe liver damage has been reported in 15% of patients with HCV infection and normal transaminase levels (14,15). Using these tests in combination is expected to have a better overall sensitivity for detecting the true disease state.

Because of the evidently equivocal correlation of the above-mentioned tests with disease activity and histopathologic findings, we need adjunctive

tests with ease of use and affordability that could obviate the need for a liver biopsy. Deposits of complement components C3 and 4 have been demonstrated in liver biopsies of patients with CHC (16). Measuring the serum levels of these proteins could give a clue about this deposition in the liver parenchyme. As immune destruction is a determinant of the liver histology, serum complement levels and biopsy findings may correlate. The aim of this prospective study was to investigate the relationship between serum complement levels, biopsy findings, viral load, and transaminase levels.

MATERIALS AND METHODS

This prospective controlled study was undertaken in the Gastroenterology Department of the Şişli Etfal Training Hospital, a tertiary teaching and research facility in İstanbul. Patients with a diagnosis of hepatitis C virus infection between 2008-2009 were enrolled. The study protocol was approved by the local ethics committee. Patients with a high sedimentation rate and/or C-reactive protein (CRP) level, proteinuria, glomerulonephritis, arthritis, cutaneous vasculitis, use of immunosuppressive drugs (glucocorticoids, cyclosporine, azathioprine, interferon, etc.), coinfection with hepatitis B and/or D, or cirrhosis in the liver biopsy were excluded from the study.

HCV Viral Load and Serology

Patients gave blood samples for HCV-RNA testing to determine viral load before the initiation of treatment. HCV-RNA quantitation was done using COBAS Taqman HCV test v2.0 (Roche Molecular Systems) in accordance with the manufacturer's instructions. Dynamic range for HCV-RNA quantitation was 6-110,000,000 copies/ml. HBsAg, anti-HCV and anti-delta testing were performed using ETI-MAK-4, AB-HCVK-4 and ETI-AB-DEL-TAK-2 kits with an ETIMAX device (DiaSorin). Serum complement levels of C3 and C4 were determined using nephelometry with an image device (Beckman-Coulter). ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), albumin, CRP, erythrocyte sedimentation rate (ESR), urinalysis, and urinary protein determination were done for every patient. The upper limits for ALT were 30 IU/L for males and 19 IU/L for females (17).

Liver Histology

Liver biopsy was performed using a Hepafix® 16G, 1.6 mm (Braun) liver biopsy needle after obtaining

informed consent from the patients. Liver biopsy samples (>1-1.5 cm in length) were stained and evaluated by a pathologist unaware of the clinical and virological results. The pathologist quantified the histological activity index (HAI) ranging from 0 to 18, and fibrosis was scored on a scale of 0 to 4 (0: no fibrosis, 1: mild fibrosis, 2: moderate fibrosis, 3: severe fibrosis, 4: cirrhosis) as described by Knodell et al. (18).

Statistical Analysis

Scale variables were presented as mean $\pm$ standard deviation (mean $\pm$ SD). Categorical data was evaluated using chi-square analysis or with Pearson's correlation as appropriate. Student's t test was used for comparison of parametric data. Curve estimation analysis was performed to determine whether a linear, logarithmic or inverse relation existed between C4 and HAI scores. Multiple group comparisons for linear data were performed using analysis of variance (ANOVA) and post-hoc Tamhane test. A p-value <0.05 was considered statistically significant. The SPSS (Statistical Package for the Social Sciences, for Windows, release 12.0.0 standard version) software was used for statistical evaluations.

RESULTS

A total of 70 patients were included in the study. According to transaminase levels, patients were divided in two groups, with patients with high transaminase level as the first group (n: 37, 20 females, 54%) and patients with normal transaminase as the second group (n: 33, 19 females, 58%). Genotype was subtype 1b in all CHC patients. An

overall comparison of patients with high and normal transaminase levels is given in Table 1. Levels of complement components C3c and C4 were significantly lower in CHC patients with high transaminase levels (p: 0.001). AST/ALT ratio was not significantly different between groups (p: 0.802).

There was no significant correlation between serum C3c and ALT (p: 0.077), AST (p: 0.070), GGT (p: 0.655), ALT/AST ratio (p: 0.284), HCV-RNA (p: 0.557), HAI (p: 0.557), or fibrosis score (p: 0.133) in patients with high transaminase levels. There was a significant correlation between serum C4 levels and ALT (r: -0.368, p: 0.001) and HAI (r: -0.639 p: 0.001), but GGT (r: -0.188 p: 0.239), ALT /AST ratio (r: -0.091, p: 0.337), HCV-RNA (r: -0.150 p: 0.398) and fibrosis (r: -0.264 p: 0.183) did not show a significant correlation. The graphic relation between serum C4 level and HAI is shown in Figure 1.

Serum ALT did not show a significant correlation with GGT (r: 0.234, p: 0.135), HCV-RNA (r: 0.007, p: 0.968), HAI (r: 0.283, p: 0.152), or fibrosis (r: -0.118, p: 0.556) in patients with high ALT levels. Serum GGT also showed no significant correlation with any of the activity markers tested.

Two patients with normal transaminase levels had a low C4 level.

DISCUSSION

Complement is a non-cellular component of the immune system. It may either cause direct cellular damage or contribute to damage via opsonization and chemotaxis-inducing effects (19). Comple-

Table 1. Comparison of CHC cases with high transaminase, low transaminase and controls

	High transaminase CHC (n=37)	Normal transaminase CHC (n=33)	Controls (n=30)	p
Age (year)	53.88 $\pm$ 11.44	51.23 $\pm$ 7.24	54.30 $\pm$ 9.47	0.470
C3c (mg/dl)	102.06 $\pm$ 27.7	118 $\pm$ 29.4	136.2 $\pm$ 15.24	0.001
C4 (mg/dl)	15.17 $\pm$ 5.66	22.55 $\pm$ 5.67	28.5 $\pm$ 3.83	0.001
ALT (U/L)	85.64 $\pm$ 47.6	21.81 $\pm$ 6.5	8.72 $\pm$ 4.04	0.001
AST (U/L)	68.48 $\pm$ 40.95	24.5 $\pm$ 5.18	15.45 $\pm$ 3.26	0.001
GGT (U/L)	62.65 $\pm$ 42.3	17.6 $\pm$ 4.5	15.3 $\pm$ 7.65	0.001
HAI	8 $\pm$ 2.75	-	-	
Fibrosis	1.66 $\pm$ 0.784	-	-	
HCV-RNA (IU/ml)	min: 6	-	-	
	max: 10 ⁶ x 106	-	-	
	4554701.53 $\pm$ 17590562	-	-	

C3c: Complement 3c. C4: Complement 4. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CHC: Chronic hepatitis C. GGT: Gamma-glutamyl transpeptidase. HAI: Histological activity index.

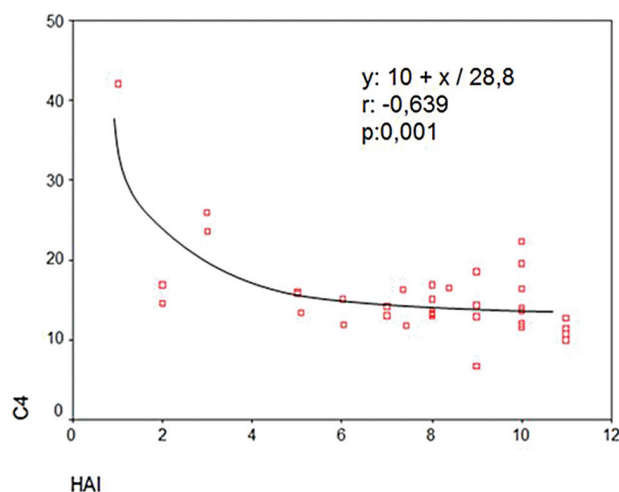


Figure 1. The inverse relation between complement C4 and histological activity index (HAI) score in CHC patients with high transaminase levels. Formula for the curve is $y = 10 + x / 28,8$, which explains the relation with 70.8% accuracy. The parabolic fall of complement from high level to the mid ranges as HAI scores increase may indicate the presence of a cofactor.

ment plays the major role in the immune destruction of the liver in patients with CHC. Complement's central role in immune destruction of hepatocytes has been well demonstrated in studies showing complement deposition in the liver biopsy of cases with chronic hepatitis (16) and a reduction in inflammation after complement system inhibition (20).

This study primarily compared complement system activation in CHC patients with normal and high transaminase levels. In addition, biopsy specimens from high transaminase patients were evaluated for a relationship between complement activation and HAI with Knodell scoring and fibrosis. Serum C3c and C4 levels in patients with high transaminase levels were significantly lower than in those with normal transaminase levels, and low serum C4 concentration showed a significant negative correlation with biopsy findings.

As in our study, Dumestre-Perard and colleagues (21) found that serum complement C4 level was low in patients with CHC. In contrast to our findings, they did not determine any relation between C4 levels and histological changes in Knodell score including HAI and fibrosis. On the other hand, we did not consider fibrosis as a component of inflammatory activity and considered that HAI in the Knodell scoring would be a more appropriate indicator of inflammation than the total score.

The finding that serum complement C4 level correlates with liver histopathology is important. The ability of the tests that are currently used in the diagnosis and follow-up of CHC patients (ALT and HCV-RNA quantity) to show histological changes are equivocal, and the current guidelines generally take into account studies indicating their poor utility in follow up. Like many other studies and guidelines, findings from our study confirm the notion that ALT, HCV-RNA and GGT are not good indicators of liver inflammation as assessed by Knodell scoring (8,22-26). The aim of using these follow-up markers is to determine disease activity and initiate, modify or withhold treatment accordingly. Serum C4 level shows an inverse relationship with HAI for Knodell scoring. Thus, we propose C4 level as a complementary test for the follow-up of patients with CHC.

The significant relationship between C4 level and HAI in patients with high transaminase levels has two main implications. First, if this relationship is confirmed and proves useful in the follow-up, it may obviate the need for a liver biopsy in certain situations. Second, it may contribute to our arsenal of follow-up markers, which overall currently have an equivocal, weak relationship with biopsy findings.

We had two patients in the normal transaminase group with low complement levels. Severe liver damage has been observed in up to 15% of CHC patients with normal transaminase levels (14,15). The relationship between low C4 levels in patients with normal ALT deserves to be investigated.

There are limitations to our study. Although the main objective was to compare C3 and C4 levels in patients with CHC having normal or high transaminase levels, obtaining more biopsy samples could have made the relationship with histology more reliable, though still statistically adequate. A multivariate modeling using more variables could have resulted in a more accurate estimate for the HAI. Although complement activation is mainly attributed to the lectin route in chronic hepatitis patients (21,27), we could have examined other components of the system to assess that hypothesis. Finally, a biopsy would have been valuable to clarify the histology of those patients with normal transaminase and low complement levels.

New studies regarding complement-mediated immune breakdown may lead towards different treatment modalities. In the study carried out by

Chang et al. (20), it was advocated that inhibition of complement may reduce the inflammation, and this may bear implications regarding new treatment modalities. Immune breakdown due to complement activation in CHB patients may be a good pathway for targeted treatments that have emerged in recent years (such as eculizumab, complement 5 monoclonal antibody) (28).

In conclusion, complement is a means of immune destruction in CHC disease. The serum complement level is lower in patients with high transaminase levels who presumably have active liver inflammation and correlates with HAI as well. Determination of complement 3 and 4 levels together with currently recommended follow-up markers (ALT and HCV-RNA) could offer additional benefit.

REFERENCES

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; 345: 41-52.
2. Negro F, Alaei M. Hepatitis C virus and type 2 diabetes. *World J Gastroenterol* 2009; 15: 1537-47.
3. Yuki N, Hayashi N, Kamada T. HCV viremia and liver injury in symptom-free blood donors. *Lancet* 1993; 342: 444.
4. He XS, Rehman B, Lopez-Labrador FX, et al. Quantitative analysis of hepatitis C virus-specific CD8(+) T cells in peripheral blood and liver using peptide-MHC tetramers. *Proc Natl Acad Sci USA* 1999; 96: 5692-7.
5. Scheurer PJ, Ashroftzadeh P, Scherlock S, et al. The pathology of hepatitis C. *J Clin Invest* 1991; 1058-60.
6. Pawlotsky JM. Use and interpretation of virologic tests for hepatitis C. *Hepatology* 2002; 36: 476.
7. Mohamadnejad M, Pourshams A, Malekzadeh R, et al. Healthy ranges of serum alanine aminotransferase levels in Iranian blood donors. *World J Gastroenterol* 2003; 9: 2322-4.
8. Strader BD, Wright T, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39: 1147-71.
9. Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; 36(Suppl 1): S21-S29.
10. Farci P, Alter HJ, Wong D, et al. A long term study of hepatitis C virus replication in non A, non B hepatitis. *N Engl J Med* 1991; 325: 98-104.
11. Puoti M, Zonaro A, Ravaggi A, et al. Hepatitis C virus RNA and antibody response in clinical course of acute hepatitis C virus infection. *Hepatology* 1992; 16: 877-81.
12. Marco LD, Gillio-Tos A, Fiano V, et al. Occult HCV infection: an unexpected finding in a population unselected for hepatic disease *PLoS ONE* 2009; 4: E8128.doi:10.1371.
13. National Institutes of Health Consensus Development Conference Panel Statement: management of hepatitis C. *Hepatology* 1997; 26(Suppl): 2S-10S.
14. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; 137: 1-10.
15. Puoti C, Castellacci R, Montagnese F, et al. Histological and virological features and follow up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of asymptomatic C carriers (ISACC). *J Hepatol* 2002; 37: 117-23.
16. Vasei M, Monabbati A, Alizadeh-Naeeni M, et al. Immunoglobulin and complement depositions in the liver of chronic hepatitis patients. *Hepatogastroenterology* 2008; 55: 1066-70.
17. Assy N, Benishvili Z, Djibre A, et al. Lower baseline ALT cut-off values and HBV DNA levels better differentiate HBeAg (-) chronic hepatitis B patients from inactive chronic carriers. *World J Gastroenterol* 2009; 15: 3025-31.
18. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. *J Hepatology* 2007; 47: 598-607.
19. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001; 344: 1058-66.
20. Chang ML, Yeh CT, Lin DY, et al. Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation. *BMC Med Genomics* 2009; 2: 51.
21. Dumestre-Perard C, Ponard D, Drouet C, et al. Complement C4 monitoring in the follow-up chronic hepatitis C treatment. *Clin Exp Immunol* 2002; 127: 131-6.
22. Choi Y, Putti T, Win K, et al. Correlation of viral RNA, alanine aminotransferase, and histopathology in hepatitis C virus-associated hepatitis. *Mol Diagn* 1999; 4: 251-4.
23. Lee YS, Yoon SK, Chung ES, et al. The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. *J Korean Med Sci* 2001; 16: 585-91.
24. Altıparmak E, Saritas U, Tureyen A, et al. Relationship between histological damage, viral load and serum transaminase levels in patients with chronic hepatitis C. *Turk J Gastroenterol* 2001; 12: 185-8.
25. Hui CK, Belaye T, Montegrande K, et al. A comparison in the progression of liver fibrosis in chronic hepatitis C between persistently normal and elevated transaminases. *J Hepatol* 2003; 38: 511-7.
26. Persico M, Persico E, Suozzo R, et al. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. *Gastroenterology* 2000; 118: 760-4.
27. Kilpatrick DC, Delahooke TE, Koch C, et al. Mannan-binding lectin and hepatitis C infection. *Clin Exp Immunol* 2003; 132: 92-5.
28. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2006; 355: 1233-43.