Impact of sustained virus elimination on natural anticoagulant activity in patients with chronic viral hepatitis C

Aida Saray*, Rusmir Mesihović, ZoraVukobrat-Bijedić, Srđan Gornjaković,
Nenad Vanis, Amila Mehmedović, Vedad Papović, Sanjin Glavaš

Department of Gastroenterology and Hepatology, Clinical Centre of Sarajevo University, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

Abstract

Previous studies have reported reduced synthesis of various hemostatic factors in patients with chronic liver disease. Whether changes in plasma levels of these proteins reflect recovered liver synthetic function following virological eradication therapy has not been approved yet. The aim of the study was to determine the impact of sustained viral suppression achieved with pegylated interferon alpha and ribavirin on hemostatic parameters including natural anticoagulants in patients with chronic hepatitis C.

The following coagulation screening tests were obtained in thirty patients with chronic viral hepatitis C before and after completion of antiviral treatment: activated partial thromboplastin time, prothrombin time, plasma fibrinogen and natural anticoagulant proteins antithrombin III, protein C (PC) and total protein S (PS) activity. Only patients who achieved durable virus suppression were included.

The mean PC and PS levels were significantly lower in patients with chronic viral hepatitis C before antiviral therapy than in healthy controls (79.04 ± 16.19 % vs. 109.92 ± 21.33% and 54.04 ± 16.11% vs. 87.60 ± 8.15%, respectively; ($p<0.001$). Mean levels of PC exhibited a significant increase by 14.69 % after the completion of antiviral treatment (93.73 ± 14.18%, $p<0.001$) as well as PS levels, which significantly increased by 21.46% (75.50 ± 15.43, $p<0.001$) when compared with pre-treatment values. No remarkable fluctuations in other hemostatic parameters were noted.

Protein C and protein S are sensitive markers of hepatocyte synthetic impairment and are valuable markers in monitoring the efficacy of antiviral treatment in chronic hepatitis C patients. Larger studies are needed to confirm our results.

INTRODUCTION

Chronic liver disease is commonly associated with complex hemostatic defects that include impaired synthesis of a number of coagulation proteins and their synthesis is variably impaired in liver disease [1, 2]. Previous studies have shown diminished circulating levels of natural anticoagulants antithrombin III (AT III), protein C (PC) and protein S (PS) in those with chronic liver disease [3,4,5] as well as acute liver disease [6] as a result of impaired liver synthetic function. In a recent study, anticoagulants were shown as sensitive markers of liver disease with a marked decrease in chronic hepatitis C patients [7]. Despite these results their role in chronic viral hepatitis is still unclear. Interferon alpha (IFN α) is a natural occurring cytokine with immunomodulatory, antiproliferative and antiviral activity [8]. IFN α has been a mainstay in the treatment of chronic hepatitis C infection and the development of pegylated interferon alpha (PEG-IFN-α) added a new milestone to the treatment in these patients due to his improved pharmacokinetic profile [9, 10]. Combination of PEG-IFN-α with ribavirin improves the overall antiviral treatment outcome and has become the standard therapy for chronic hepatitis C infection. The goal of antiviral therapy is to achieve sustained elimination of the virus, which is accompanied by reduction of hepatitis virus related morbidity and mortality [11]. Several studies have demonstrated the virological, biochemical and histological effects of PEG-IFN-α/ribavirin combination therapy; however, hemostatic effects are not well studied before.

* Corresponding author: Aida Saray,
Department of Gastroenterology and Hepatology, Medical University Center Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
Phone: +38733 297913;
Fax: +38733 297071;
e-mail: sarayaida19@gmail.com

In the present study we determined conventional coagulation screening tests including anticoagulant activity in patients with hepatitis C before and following antiviral therapy termination. We aimed to study whether sustained virus elimination following combination therapy PEG-IFN-α and ribavirin has an impact on coagulation proteins and anticoagulant activity in patients with chronic viral hepatitis C which might reflect improved liver synthetic function due to successful virus suppression.

MATERIALS AND METHODS

Patients

Thirty-three consecutive patients referred to the Department of Gastroenterology and Hepatology of the Medical Center University of Sarajevo for chronic hepatitis C infection from November 2007 to march 2010 were recruited. Three individuals were excluded from the study, one because of discontinuation of antiviral treatment due to serious side effects and two patients did not achieve negative HCV-RNA at the end of antiviral treatment. All patients included in the study achieved sustained virological response (SVR), defined as negative HCV-RNA six months after the end of antiviral treatment. The diagnosis of chronic viral hepatitis was based on biochemical tests, positive RNA PCR assays and confirmed by liver biopsy in all patients before the initiation of antiviral treatment. Histological changes of chronic hepatitis were evaluated according to the classification system proposed by Ishak and colleagues [12]. All the patients were treated with pegylated interferon alpha (18 patients received PEG-IFN-α 2a at a fix dose of 180μg and 12 patients were treated with 1.5μg/kg PEG-IFN-α 2b) subcutaneously once weekly plus weight-based ribavirin (800-1200 mg ribavirin daily). According to the accepted treatment protocol for chronic hepatitis C infection patients with HCV genotype 1 and 4 were treated 48 weeks (n=20) and patients with HCV genotype 2 and 3 (n=10) were treated 24 weeks with antiviral therapy. Exclusion criteria were the following: previous antiviral or immunomodulatory therapy, hepatocellular carcinoma or other known malignancy, other chronic liver disease, hepatitis B and C virus co-infection, history of deep venous thrombosis, and current anticoagulation therapy. Patients who 6 months after treatment termination did not achieve viral suppression defined by undetectable PCR-assay were excluded from the study. A control group consisted of 30 healthy individuals with normal results of physical examination and laboratory blood findings were selected from the general public. Their basic epidemiological and laboratory parameters are summarized in Table 1.

Procedures

The Ethical Committee of the Medical Centre University of Sarajevo approved the study protocol and informed consent was taken from all patients before undergoing liver biopsy. Coagulation screening tests were obtained on day 1 of admission to our department (pre-treatment sample) and six months after successful completion of antiviral eradication therapy (post-treatment sample).

Coagulation Assays

Blood samples were collected by venipuncture directly into vacuum tubes containing trisodium citrate. The blood samples tubes were centrifuged at 2.000 x g for 15 min at room temperature. The assays were performed on fresh plasma or on aliquots, which were immediately stored at -70ºC until analysis was performed. Blood samples were obtained, from each patient on the liver biopsy date and after PEG-IFN-α treatment termination. Coagulation screening tests included: activated partial thromboplastin time (aPTT) and prothrombin time expressed as international normalized ratio (INR) were performed by the conventional methods. Plasma fibrinogen was measured by the turbidometric method of Clauss (Dade Thrombin Reagent) [13]. Natural anticoagulants were assayed using commercial reagent kits (Dade Behring, Marburg, Germany) according to the manufacturer's instructions: Activities of AT III were determined by colorimetric assay (Berichrom AT III), PC activity by kinetic testing (Berichrom C) and PS activity (Protein Sac) by a clotting assay. Anticoagulant activity was expressed in % with a reference range of 75 − 125% for AT III, 70 − 140% for PC, and 60-130% for PS.

Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using the Student t-test to compare the means of independent groups. Logarithmic transformation was applied to the group of data that did not show normal distribution to reduce variances before applying t-test using the new transformed values. A two-tailed *p*-value below 0.05 was considered to be statistically significant. Statistical analysis was performed using the statistical package SPSS 16.0.

RESULTS

Baseline characteristics of healthy controls (n=30) and patients with chronic hepatitis C (n=30) are presented in Table 1. The mean age was not significantly different across the study groups. Patients with chronic hepatitis C had significantly higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-

TABLE 1. Demographic and biochemical characteristics of healthy controls and patients with chronic hepatitis C

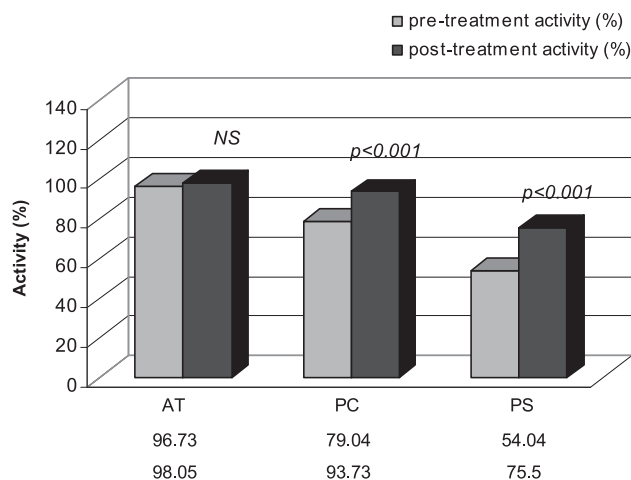|	*Chronic hepatitis C* *n= 30*	*Contro* *n= 30*	*p*
Sex (M/F)	16/14	17/13	NS
Age (years)	45.93 ± 10.70	42.17 ± 10.83	NS
Haemoglobin (g/l)	148.27 ± 14.75	151.81 ± 10.91	NS
Platelet count (x10³/l)	206.71 ± 71.91	251.48 ± 50.11	*p*<0.001
ALT (IU/l)	102.42 ± 66.40	24.04 ± 8.82	*p*<0.001
AST (IU/l)	62.44 ± 49.86	24.19 ± 7.87	*p*<0.001
GGT (IU/l)	53.22 ± 52.61	27.89 ± 9.90	*p*=0.027
Albumin (g/l)	40.77 ± 4.72	41.13 ± 4.07	*NS*

Legend: Quantitative values are expressed as mean (SD), ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT gamma-glu-tamyl-transpeptidase;

TABLE 2. Results of coagulation assays in chronic hepatitis C patients treated with PEG-IFN-α / ribavirin

	Pre-treatment	Post-treatment	Control
Fibrinogen (g/dl)	3.35 ± 1.01	3.37 ± 0.75	3.36 ±0.61
APTT(s)	31.80 ± 3.63	31.24 ± 2.70	30.83± 2.10
INR	1.09 ± 0.10 *p=0.005	1.05 ± 0.08 **p=0.044	1.03 ± 0.12
AT III (%)	96.73±14.27	98.05 ± 12.03	99.80 ± 13.93
PC (%)	79.04 ± 16.19 *p<0.001	93.73 ± 14.18 **p<0.001 ***p<0.001	109.92 ± 21.33
PS(%)	54.04 ± 16.11 *p<0.001	75.50 ± 15.43 **p<0.001 ***p<0.001	87.60 ± 8.15

Legend: Quantitative values are expressed as mean ± standard deviation, APTT: activated partial thromboplastin time, INR: international normal-ized ratio, AT: antithrombin, PC: protein C, PS: protein S; *significant difference between pretreatment sample and controls; **significant difference between pretreatment sample and post-treatment sample; ***significant difference between post-treatment sample and controls

glutamyl-transpeptidase (GGT) compared to healthy controls. Mean platelet count was significantly lower in chronic hepatitis patients. Mean hemoglobin and albumin levels showed no significant difference (Table 1). The mean fibrinogen level showed no significant difference in patients with chronic hepatitis C infection (3.35 ± 1.01 g/l) when compared with normal control levels (3.36 ±0.61 g/l). No fluctuations of fibrinogen levels were achieved following antiviral therapy. Prolongation of INR was noted before treatment commencement (1.09±0.10) when compared to controls (1.03±0.12, p=0.005) and shortened after treatment cessation (1.05±0.08, p=0.044) compared to pre-treatment values and showed no significant difference to healthy controls. APTT levels did not show remarkable alterations (Table 2). The mean PC (79.04 ± 16.19%) and PS (54.04 ± 16.11%) levels were significantly lower in patients with chronic viral hepatitis C before antiviral treatment than in the healthy control group (109.92 ± 21.33, p<0.001). At the same time the mean level of AT III in the pre-treatment group (96.73±14.27%)

FIGURE 1. Pre- and post-treatment anticoagulant activity in chronic hepatitis C patients
Legend: AT – antithrombin; PC protein C, PS- protein S

showed no significant reduction in comparison with controls (99.80 ± 13.93). The levels of PC exhibited a significant increase after the completion of PEG IFN-α/ribavirin therapy (93.73 ± 14.18%) when compared with pre-treatment values (79.04 ± 14.27, p<0.001) but were still statistically lower than normal control levels (109.92 ± 21.33, p<0.001). Mean total PS values were also statistically significant increased in the post-treatment group than in those before PEG-IFN-α/riba-virin therapy (75.50 ± 15.43, p<0.001). The PS values were still statistically different from controls (87.60 ± 8.15, p<0.001). On the other hand, AT III levels showed no significant alterations caused by virus elimination (Table 2, Figure 1).

DISCUSSION

The liver plays a major role in hemostasis and it is well known that patients with chronic liver disease show a marked decrease in liver synthesis of coagulation factors as well as inhibitors [14]. Coagulation indices because of their relationship to liver synthetic function are well established as prognostic markers in a variety of settings in both acute and chronic liver disease [15, 16]. Recently, anticoagulation proteins were approved not only to reflect hepatocyte impairment [4, 5, 7] but also to have predictive value in chronic liver disease [17]. It is therefore justified to assume that diverse coagulation assay reflect hepatocyte damage in chronic viral hepatitis C, but the impact of PEG-IFN-α/ribavirin combination therapy on these hemostasis parameters have not been well studied yet. The current study is an attempt to prove whether hemostatic parameters are useful markers in monitoring antiviral treatment in chronic hepatitis C patients. We studied exclusively patients who achieved response to PEG-IFN-α/ribavirin therapy de-

fined by durable suppression of HCV RNA to low or undetectable levels after treatment cessation. The present study shows in untreated patients with chronic hepatitis C significant reduction in plasma levels of PC and PS. These findings are in line with previous studies, which demonstrated markedly reduced anticoagulant activity in chronic hepatitis C patients as a sign of reduced hepatocyte synthetic capacity [7, 17]. A recent study from Italy even showed that in chronic liver disease reduction in plasma levels of PC and PS correlated with a higher model for end-stage liver disease (MELD) score [18]. Another group of investigators studied 154 patients with chronic liver disease and approved PS and PC as predictors of hepatic inflammation and fibrosis [17]. These findings taken together confirm that levels of PC and PS are sensitive markers of hepatocyte impairment. At the same time, we noted AT III levels not reduced in chronic hepatitis C patients in comparison with healthy controls and this could be interpreted as AT III to be a less sensitive protein reflecting hepatocyte malfunction. AT III was shown to be a good marker of liver cell synthetic function in cirrhosis [19, 20, 21], acute hepatitis [7, 22] as well as HCC [23, 24], but in chronic viral hepatitis C AT III did not show any marked reduction [7]. Furthermore the fact that AT III was not different between the patients with chronic hepatitis C and normal controls emphasizes the reduction of PC and PS levels as the earliest disturbance of the coagulation system in chronic liver disease as suggested by few previous investigators [7, 17]. After completion of antiviral treatment, we found a significant increase in total PC and PS values. This increase could be attributed to improved hepatocyte synthetic function following successful antiviral treatment. These findings are in contrast with one study where an increase of total PS values but not in free PS as well as PC levels in response to standard IFN were found [25]. The authors presumed that IFN therapy has an impact on concomitant increase in liver synthesis of PS as well as of its carrier protein C4BP that is also synthesized by the liver, but not in PC. However, that study was performed on a less number of patients and it was not pointed out if anticoagulant levels were measured after achieving viral suppression. The alteration of PC levels due to antiviral therapy we attributed to recovered synthetic function in response to durable viral replication suppression after completion of PEG-IFN-α/ribavirin therapy. Although prolongation of conventional coagulation screening tests is a known feature in chronic liver disease [26, 27], no significant difference in aPTT and fibrinogen levels were noted in untreated as well as treated patients as compared with controls. This is in concordance with previous investigations [5, 7, 25].

The prothrombin time (PT) and its derived measures of international normalized ratio (INR) are measures of the extrinsic pathway of coagulation and its effected by changes in levels of factor I, II, V, VII and X. Factor VII is the first coagulation protein to decrease when there is a hepatocyte damage, probably because of its short half-life (2-4h) [28,29]. In advanced liver disease, there is a correlation between PT prolongation and levels of F VII [30]. In the current study significant prolongation of PT, expressed as INR, in chronic hepatitis C patient should be attributed to reduction of F VII levels. It is possible that the significant shortening of PT observed following PEG-IFN-α/ribavirin therapy reflects improvement of hepatic synthetic function and normalization of F VII levels. For confirmation of these hypothesis studies evaluating the F VII level in chronic hepatitis C patients are needed.

CONCLUSIONS

On the basis of our results we concluded that durable viral suppression obtained by PEG-IFN-α/ ribavirin combination therapy in chronic hepatitis C has an impact on PC and PS activity reflecting improved liver synthetic function following sustained elimination of hepatitis C virus. The present study provided evidence supporting the hypothesis that natural anticoagulants PS and PC are sensitive markers of hepatocyte synthetic impairment and is valuable markers in monitoring the efficacy of antiviral eradication in chronic hepatitis C patients. However, the study results need to be confirmed by future studies with larger sample size.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Mammen EF. Coagulation abnormalities in liver disease. Hematol Oncol Clin North Am 1992;6(6):1247-1257.

[2] McCormick PA, Murphy KM. Splenomegaly, hypersplenism and coagulation abnormalities in liver disease. Baillieres Best Pract Res Clin Gastroenterol. 2000;14(6):1009-1031.

[3] De Caterina M, Tarantino G, Farina C, Arena A, di Maro G, Esposito P, Scopacasa F. Haemostasis unbalance in Pugh-scored liver cirrhosis: characteristic changes of plasma levels of protein C versus proteinn S. Haemostasis 1993; 23:229-235.

[4] Kujovich JL. Haemostatic defects in end stage liver disease. Crit Care Clin 2005;21:563-87

[5] Gürsoy S, Başkol M, Torun E, Yurci A, Soyuer I, Eser B. et al. Importance of anticoagulant proteins in chronic liver diseases. Turk J Gastroenterol. 2005; 16(3):129-133.

[6] O'Grady JG, Langley PG, Isola LM, Aledort LM, Williams R. Coagulopathy of fulminant hepatic failure. Semin Liver Dis. 1986; 6(2):159-163.

[7] Al Ghumlas AK, Abdel Gader AG, Al Faleh FZ. Haemostatic abnormalities in liver disease: could some haemostatic tests be useful as liver function tests? Blood Coagul Fibrinolysis. 2005; 16: 329-335.

[8]   Sen GC. Viruses and interferons. Annu Rev Microbiol. 2001; 55:255-281.

[9]   Lawrence SP. Advances in the treatment of hepatitis C. Adv Intern Med. 2000; 45:65-105.

[10] Liaw YF. Management of patients with chronic hepatitis B. J Gastroenterol Hepatol. 2002; 17(4):406-408.

[11] Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007; 147(10):677-84

[12] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F. et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995; 22(6):696-699.

[13] Clauss A. Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 1957; 17:237-246.

[14] Tripodi A, Mannucci PM. Abnormalities of hemostasis in chronic liver disease: reappraisal of their clinical significance and need for clinical and laboratory research. J Hepatol. 2007; 46(4): 727-733.

[15] Polson J, Lee WM, American Association for the Study of Liver Disease. AASLD position paper: the management of acute liver failure. Hepatology 2005;41(5):1179-1197.

[16] Schepke M, Roth F, Fimmers R, Brensing KA, Sudhop T, Schild HH. et al. Comparison of MELD, Child-Pugh, and Emory model for the prediction of survival in patients undergoing transjugular intrahepatic portosystemic shunting. Am J Gastroenterol. 2003;98(5):1167-1174.

[17] Abdo AA, Sanai FM, Azzam N, Al Sawat K, Al Dukhayil M, Al Ghumlas A, Hersi A. et al. Natural anticoagulants can be useful predictors of severity in chronic liver disease. Blood Coagul Fibrinolysis. 2010;21(2):122-127.

[18] Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F. et al. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. J Hepatol. 2009; 51(4): 682-689.

[19] Raya-Sánchez JM, González-Reimers E, Rodríguez-Martín JM, Santolaria-Fernández F, Molina-Pérez M, Rodríguez-Moreno F. et al. Coagulation inhibitors in alcoholic liver cirrhosis. Alcohol. 1998; 15(1):19-23.

[20] Kłoczko J, Mian M, Wojtukiewicz MZ, Babiuch L, Bielawiec M, Galar M. Plasma protein C as a marker of hepatocellular damage in alcoholic liver disease. Haemostasis. 1992; 22(6): 340-344.

[21] Dumontier I, Alhenc-Gelas M, Chatellier G, Brenet P, Aiach M, Petite JP. Changes in levels of blood coagulation inhibitors in cirrhosis. Prospective study in 33 patients. Gastroenterol Clin Biol. 1992; 16(2):120-125.

[22] Bell H, Odegaard OR, Andersson T, Raknerud N. Protein C in patients with alcoholic cirrhosis and other liver diseases. J Hepatol. 1992;14(2-3):163-167

[23] Park CJ, Cho HI, Kim SI. A study on changes of coagulation inhibitors and fibrinolysis inhibitors in patients with liver cirrhosis and hepatoma. J Korean Med Sci. 1991; 6(1):1-6.

[24] Kato S, Kawasaki H. Blood coagulation and fibrinolysis in relation to endotoxemia in liver cirrhosis and hepatocellular carcinoma. Nihon Shokakibyo Gakkai Zasshi. 1995; 92(8):1143-1148.

[25] Al Ghumlas AK, Abdel Gader AG, Al Faleh FZ. Natural anticoagulants and fibrinolytic activity following interferon therapy in chronic viral hepatitis. Blood Coagul Fibrinolysis. 2008; 19: 263-267.

[26] Deitcher SR. Interpretation of the international normalized ratio in patients with liver disease. Lancet 2002; 359: 47-48.

[27] Mammen EF. Coagulation defects in liver disease. Med Clin North Am. 1994; 78(3):545-554.

[28] Amitrano L, Guardascione MA, Brancaccio V, Balzano A. Coagulation disorders in liver disease. Semin Liver Dis. 2002; 22(1):83-96.

[29] Caldwell SH, Hoffman M, Lisman T, Macik BG, Northup PG, Reddy KR, et al.;Coagulation in Liver Disease Group. Coagulation disorders and hemostasis in liver disease: pathophysiology and critical assessment of current management. Hepatology 2006; 44(4): 1039-1046

[30] Bernstein DE, Jeffers L, Erhardtsen E, Reddy KR, Glazer S, Squiban P., et al. Recombinant factor VIIa corrects prothrombin time in cirrhotic patients: a preliminary study. Gastroenterology. 1997; 113(6):1930-1937.