Prothrombin time in patients with and without fibrotic chronic liver disease
M Sajjadieh, L Viunytska

INTRODUCTION
Prothrombin time (PT) is a universal indicator of the severity of liver disease and is determined by vitamin K coagulation factors and fibrinogen. PT is used in prognostic models of survival and is a key criterion for acute liver failure [1]. Reverter concluded that the PT is a simple, inexpensive, quantitative, and accurate prognostic marker of liver impairment [2]. PT results are reported in seconds, as prothrombin ratios (PTR) expressed as percentages, and as international normalized ratios (INR). However, variability in thromboplastin reagents used to measure PT leads to large differences in results from different laboratories, producing considerable confusion when studies from various centers are compared [3]. This variability also creates problems in monitoring patients with advanced liver disease. The PT-INR provides a robust measure of liver function, but recent data showed INR inter-laboratory variability. The INR is used to calculate end stage liver disease scores, which are used to prioritize patients for liver transplantation [4]. The extrapolation of PT to INR is really only valid for patients stably anticoagulated with vitamin K antagonists and may not be valid for patients with liver disease [5].

The aim of this study was to determine PT, PTR and INR in liver disease and assess the correlation between each test and clinical characteristics that are frequently assessed in the management of liver disease.

MATERIALS AND METHODS
The present study included 60 subjects who were 30 years of age or older. The patients were categorized into three groups: 20 patients with chronic hepatitis C without fibrosis (group I), 20 patients with cirrhosis with different fibrosis (group II), and 20 healthy participants (group III [control]). None of the patients were receiving anticoagulant therapy at the time of the study. The local ethics committee approved this study. All samples that were collected in the Department of Gastroenterology of the Shalimov Institute (Kiev, Ukraine) were tested for PT in a biochemical laboratory. Hepatic infection in group I was confirmed by enzyme linked immunosorbent assay (ELISA) and absence of fibrosis were confirmed by a biochemical marker of fibrosis, such as platelet count, activity of GGT, or concentration of serum cholesterol [6] and marker of fibrosis was confirmed by liver biopsy (Ishak Fibrosis Score). Plasma was obtained from fasting blood samples drawn by venipuncture with Vacutainer® tubes: 4.5 mL blood was drawn into tubes containing 0.5 mL sodium citrate 3.8%. Samples were centrifuged at 1000 (rcf) for 15 minutes, plasma was removed to plastic tubes using a plastic pipette and immediately analyzed for PT. PT was measured by the Quick method [7] with a reactive test-standard (P. Z.
Cormay, Lublin, Poland) on automated coagulograph (K-3002 Optik-Poland), the one stage prothrombin measures the clotting time of test plasma after addition of a thromboplastin reagent (International Sensitivity Index [ISI], 1.2) containing calcium chloride. The INR was calculated by raising the PTR (prothrombin time of patient over mean prothrombin time of normal subjects) to the power of the ISI of the thromboplastin reagent. PTR was calculated using the patient’s PT divided by the mean normal PT, multiplied by 100, to convert to a percentage.

Data are expressed as means ± standard deviation (SD). Statistical significance between individual samples was determined by a two tailed t test, and correlations between various measurements were assessed. Statistical analysis was performed using the SPSS 16 (SPSS, Chicago, IL, USA) for Microsoft Windows XP Professional (Microsoft, Seattle, WA, USA). P<0.05 was considered statistically significant.

RESULTS

Group I consisted of 8 male and 12 female patients with a mean age of 44.8 years (range 35-60 years); group II consisted of 11 male and 9 female patients with a mean age of 43 years (range 38-63 years); and group III consisted of 10 male and 10 female patients with a mean age of 45.8 years (range 38-52 years). PT, PTR, and INR for each group are presented in Table 1. The physiologically normal range for PT, based on reference values from the laboratory, is 12-14 sec.

PTs and INR parameters were significantly different between patients and control group. The mean PTR did not significantly differ between study groups and group III. The mean INR was only significantly differed between groups I and II.

Table 2 shows statistical tests of means against reference constants in each study group.

DISCUSSION

PT is incorporated in almost all the prognostic models for liver disease used worldwide and used in making major therapeutic decisions, such as those regarding liver transplantation in acute liver failure and cirrhosis, or regarding steroid therapy in alcoholic hepatic [8910]. Liver
Prothrombin time in patients with and without fibrotic chronic liver disease

Prothrombin time (PT) and International Normalized Ratio (INR) are used to assess coagulopathy and the prognosis for fibrosis. Nevertheless, PT does not adequately reflect coagulation abnormalities in patients with chronic liver disease. However, it is of the utmost importance to realize that both PTR and INR have substantial intra-laboratory variation in these patients [10]. The intra-laboratory variation of these tests is well known and was shown by Robert et al. also to exist in patients with liver failure [13]. Kovacs et al. concluded that different reagents that act as thromboplastin do not result in the same INR from the same samples [14].

According to other studies, the Quick method, which we used for determining PT, may result in mistakes. Based on the opinion of Horsti, the Owren PT method rather than the Quick PT method improves INR measurement in patient care [15]. While our method did not perform as well as the Owren PT method, this distinction may be unimportant, because the INR has been reported in several studies to be unsuitable for standardization in patients with liver disease [16,19]. Instead of reflecting most injuries to liver cells, INR is typically abnormal only in patients with advanced liver disease. This test is not sensitive enough to detect a minor impairment in liver function. Recently, two studies incorporated INR in different models to predict significant fibrosis in patients with hepatitis C [17,18].

Our data have indicated that only INR were significantly different between groups I and II (P<0.001), and, thus, a marker for the absence or presence of fibrosis in chronic liver disease. However, a new INR specific for liver diseases (INR “LD”) be more helpful than INR in this setting, as INR LD could be used to standardize PT in liver disease, according to Laurent et al. [19].

For these reasons, PT is a quantitative and accurate prognostic marker of liver impairment in the presence of hepatic fibrosis. However, in the future, there should be an attempt to either standardize the PTs and/or INR in these patients or identify a better marker of synthetic function of the liver.

CONCLUSION

We found PT and INR were significantly different between patients cirrhosis and chronic hepatitis without fibrosis, but PTR not significantly different between these patients.

disease, vitamin K deficiency, warfarin intoxication, and primary fibrinolysis may be associated with prolonged PT [1]. Virtually all patients with advanced liver disease have coagulopathies. In all liver disease, PT is just a measurement of synthetic liver function, but in chronic liver diseases specifically, the PT is usually not elevated until cirrhosis is present and the liver fibrosis is fairly significant [2]. In addition, in advanced liver disease there is a strict balance of the procoagulant and the anticoagulant factors; anticoagulant proteins such as antithrombin III inhibit thrombin generation and keep a check on continuing clot formation. PT measures only the formation of fibrin from thrombin and does not assess the effect of fibrinolytic factors [3]. This is despite the fact that hyper-fibrinolysis is frequently reported in patients with chronic liver disease [4]. Thrombin generation is a dynamic process, in which the anticoagulant system and inhibitors of the tissue factor pathway continuously neutralize the coagulation enzymes. PT is recognized as an accurate predictor of liver damage and the likelihood of progression to end stage liver failure. It has thus been incorporated into commonly used prognostic indices of chronic liver disease such as the Child–Pugh and Mayo End-Stage Liver Disease (MELD) scores [5,6].

In our study, PT values in patients with chronic hepatitis C without fibrosis were significantly different with to those in healthy participants (P<0.01). On the other hand, PT values were significantly different in patients with cirrhosis than in healthy participants (P<0.001). A given value of PT expressed in seconds may have little meaning in geographical areas where chronic hepatitis is prevalent, because even the “normal” reference values used by laboratories in these areas will be not true as a result of impaired synthetic liver function in the population. In our study, patients with elevated biochemical fibrosis markers had PT values that differed significantly from those of control participants [7]. In addition, patients with chronic hepatitis tend to have cirrhosis leading to decreased levels of AT III [8], a marker that we suggested for fibrosis. As shown by Thachil [9] on hemostasis testing in chronic liver disease, PT does not adequately reflect coagulation abnormalities in patients with chronic hepatitis C. Nevertheless, this parameter does give a good estimate of the synthetic function of the liver and, thus, may be used as a prognostic marker for fibrosis.

The PT and INR are used to assess coagulopathy and the severity of hepatic tissue damage in the Child–Pugh score [10]. Our data indicate that INR differed significantly between groups II and III (Table 2). On the other hand, INR differed significantly between groups I and II, PTR does not adequately reflect coagulation abnormalities in patients with chronic liver disease. However, it is of the utmost importance to realize that both PTR and INR have substantial intra-laboratory variation in these patients [10].

In our study, PT values in patients with chronic hepatitis C disease, vitamin K deficiency, warfarin intoxication, and primary fibrinolysis may be associated with prolonged PT [1]. Virtually all patients with advanced liver disease have coagulopathies. In all liver disease, PT is just a measurement of synthetic liver function, but in chronic liver diseases specifically, the PT is usually not elevated until cirrhosis is present and the liver fibrosis is fairly significant [2]. In addition, in advanced liver disease there is a strict balance of the procoagulant and the anticoagulant factors; anticoagulant proteins such as antithrombin III inhibit thrombin generation and keep a check on continuing clot formation. PT measures only the formation of fibrin from thrombin and does not assess the effect of fibrinolytic factors [3]. This is despite the fact that hyper-fibrinolysis is frequently reported in patients with chronic liver disease [4]. Thrombin generation is a dynamic process, in which the anticoagulant system and inhibitors of the tissue factor pathway continuously neutralize the coagulation enzymes. PT is recognized as an accurate predictor of liver damage and the likelihood of progression to end stage liver failure. It has thus been incorporated into commonly used prognostic indices of chronic liver disease such as the Child–Pugh and Mayo End-Stage Liver Disease (MELD) scores [5,6].

In our study, PT values in patients with chronic hepatitis C without fibrosis were significantly different with to those in healthy participants (P<0.01). On the other hand, PT values were significantly different in patients with cirrhosis than in healthy participants (P<0.001). A given value of PT expressed in seconds may have little meaning in geographical areas where chronic hepatitis is prevalent, because even the “normal” reference values used by laboratories in these areas will be not true as a result of impaired synthetic liver function in the population. In our study, patients with elevated biochemical fibrosis markers had PT values that differed significantly from those of control participants [7]. In addition, patients with chronic hepatitis tend to have cirrhosis leading to decreased levels of AT III [8], a marker that we suggested for fibrosis. As shown by Thachil [9] on hemostasis testing in chronic liver disease, PT does not adequately reflect coagulation abnormalities in patients with chronic hepatitis C. Nevertheless, this parameter does give a good estimate of the synthetic function of the liver and, thus, may be used as a prognostic marker for fibrosis.

The PT and INR are used to assess coagulopathy and the severity of hepatic tissue damage in the Child–Pugh score [10]. Our data indicate that INR differed significantly between groups II and III (Table 2). On the other hand, INR differed significantly between groups I and II, PTR does not adequately reflect coagulation abnormalities in patients with chronic liver disease. However, it is of the utmost importance to realize that both PTR and INR have substantial intra-laboratory variation in these patients [10]. The intra-laboratory variation of these tests is well known and was shown by Robert et al. also to exist in patients with liver failure [13]. Kovacs et al. concluded that different reagents that act as thromboplastin do not result in the same INR from the same samples [14]. Denson et al. compared INR values in 20 patients with liver impairment, using human and rabbit thromboplastin, and reported a 25% difference in INR between the two reagents [15].

According to other studies, the Quick method, which we used for determining PT, may result in mistakes. Based on the opinion of Horsti, the Owren PT method rather than the Quick PT method improves INR measurement in patient care [15]. While our method did not perform as well as the Owren PT method, this distinction may be unimportant, because the INR has been reported in several studies to be unsuitable for standardization in patients with liver disease [16,19]. Instead of reflecting most injuries to liver cells, INR is typically abnormal only in patients with advanced liver disease. This test is not sensitive enough to detect a minor impairment in liver function. Recently, two studies incorporated INR in different models to predict significant fibrosis in patients with hepatitis C [17,18].

Our data have indicated that only INR were significantly different between groups I and II (P<0.001), and, thus, a marker for the absence or presence of fibrosis in chronic liver disease. However, a new INR specific for liver diseases (INR “LD”) be more helpful than INR in this setting, as INR LD could be used to standardize PT in liver disease, according to Laurent et al. [19].

For these reasons, PT is a quantitative and accurate prognostic marker of liver impairment in the presence of hepatic fibrosis. However, in the future, there should be an attempt to either standardize the PTs and/or INR in these patients or identify a better marker of synthetic function of the liver.

CONCLUSION

We found PT and INR were significantly different between patients cirrhosis and chronic hepatitis without fibrosis, but PTR not significantly different between these patients.
Attempts have been made to evaluate the role of PT in the management of the most frequent clinical problems of patients with severe liver disease. However, PT is poorly standardized in patients with chronic hepatitis C without fibrosis. Further research is needed to standardize these markers in various types of chronic liver disease, as they are used in staging disease, deciding on invasive procedures, and prioritizing transplantation.

ACKNOWLEDGMENTS

We thank the staff of the biochemical Laboratory at the Department of Gastroenterology of the Shalimov Institute for their assistance

CORRESPONDENCE TO

Sheikh Sjjadieh,M.R Department of clinical immunology,: Draizera Ave, 7, Kiev, Ukraine Phone: 8067-810-5445, 00380-44-440-96-80 FAX: 00380-44-456-90-27 Email:mohammad_esfahan@yahoo.com

References

Author Information

M.R. Sheikh Sajjadieh
Ph.d student of clinical immunology, National Medical Academy for Post Graduate Education

L.V. Viunytska
Associated professor of clinical biochemistry, Department of Clinical Laboratory Diagnosis, National Medical Academy for Post Graduate Education