Evaluation of Plasma D-dimer Level in Patients with Chronic Liver Disease

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Background & Study Aim: To evaluate the relationship between the presence of ascites and hyperfibrinolytic state in cirrhotic patients by measuring the circulating levels of D-dimer in those with and without ascites and to assess the effect of ascitic fluid paracentesis on the concentration of D-dimer **Patients and Methods:**This study was performed on 100 chronic liver disease patients: Group(A):50 patients with ascites and Group(B):50 patients without ascites.Both groups had laboratory investigations, abdominal ultrasonography.**Results:** In Group A,D-dimer mean level(3.3 ± 2 mg/L) in Group B, D-dimer mean level(1.5 ± 1 mg/L).In Group A after ascitic fluid paracentesis, the mean D-dimer values returned to normal range in 30 patients, decreased to the high normal level in 10 patients and decreased but remained high above normal level in 10 patients.In Group A, the D-dimer values after ascitic fluid paracentesis were not significantly different from those found in patients without ascites; Group B. The plasma D-dimer levels were highly significantly elevated in patients with HCC.Plasma D-dimer was highly significantly positively correlated with AFP.The cutoff value of plasma D-dimer for detection of HCC was 3.2, sensitivity was 80% and specificity was 82.9% Plasma D-dimer was found to be a better negative than positive test with higher specificity than sensitivity **Conclucion:**High D-dimer is associated either with presence of ascites or HCC.In patients with liver cirrhosis, high D-dimer levels in absence of ascites require more careful monitoring for HCC with a cutoff value of 3.2 mg/L for detection of HCC.

[Wesam A. Ibrahim, Sara Abdelhakam, Ameer Helmy, Hatem Abd El Lateef, Khalid A. El Wahaab, Mahmoud O. Abdelmageed, Doaa G. Eissa. **Evaluation of Plasma D-dimer Level in Patients with Chronic Liver Disease.** Researcher, 2011;3(10):13-18]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher.

Key words: Plasma D-Dimer; Chronic Liver Disease; Ascites; Hepatocellular Carcinoma

Introduction:

The process of fibrinolysis involves the conversion of plasminogen to plasmin by tissue plasminogen activator (T-PA). The plasmin formed has a specific binding site for fibrin and its primary action is to degrade fibrin clots. The degradation products are called fibrin degradations products (FDPs) (normal value: less than 8 u/ml). Under normal circumstances, FDPs are removed from the blood by the liver, kidney, and reticuloendothelial system. If the FDPs are produced at a rate that exceeds their normal clearance, they will accumulate. In high concentrations, FDPs act as anticoagulants through impairing platelet function, inhibiting thrombin, and preventing the cross-linkage of fibrin strands and lead to bleeding as seen in disseminated intravascular coagulopathy (DIC). [(1)]

Increased levels of circulating plasmin causes clot lysis and degradation of fibrinogen to the soluble fibrin monomers. Plasmin cleaves fibrinogen into fragments X, Y, D and E which are known as fibrinogen degradation products (FDPs). Plasmin also cleaves insoluble cross-linked fibrin polymers into x-oligomers. The main x-oligomers are known as D-dimers. [(2)]

D-dimers indicate the activity of both thrombin and plasmin and are specific for fibrinolysis. They are effective in detecting both intravascular and extravascular cross-linked fibrin by-products. [(1)]

It has been previously reported that ascites plays a role in causing fibrinolysis in cirrhotic patients. Ascitic fluid is essentially an ultrafiltrate of plasma in patients with portal hypertension, thus it contains coagulation-relevant proteins. If the ascites compartment is a site at which fibrinolytic activity arises, then ascites may contribute to the exaggerated fibrinolysis and bleeding tendency typically found in advanced liver disease. [(3)]

Aim of the work:

To evaluate the relationship between the presence of ascites and hyperfibrinolytic state in cirrhotic patients by measuring the circulating levels of D-dimer in those with and without ascites and to assess the effect of ascitic fluid paracentesis on the concentration of D-dimer.

Patients and Methods:

This study was performed on 100 chronic liver disease patients attending Gastroenterology and Hepatology Outpatient Clinics and admitted to

Internal Medicine and Tropical Medicine Departments of Ain Shams University Hospitals, during the period from December 2009 to December 2010. They were divided into two groups; **Group(A)** included 50 chronic liver disease patients with ascites and **Group(B)** included 50 chronic liver disease patients without ascites. Informed consent was obtained from all patients and also the approval of the ethical committee was done. Diagnosis of patients with chronic liver disease in this study was based on clinical, laboratory and ultrasonographic criteria. Patients with either moderate or tense ascites due to chronic liver disease that did not have any contraindications for paracentesis were included. Patients with minimal ascites, thrombosis in any of major intra-abdominal vessels or lower limb DVT and those with evidence of spontaneous bacterial peritonitis, TB peritonitis, hepatic encephalopathy or associated major co-morbid illness were excluded from the study.

All patients were subjected to the following:

Detailed history, thorough clinical examination, and laboratory investigations including: Complete Blood count (CBC), liver and kidney function tests, in addition to measurement of prothrombin time, alphafetoprotein and D-dimer concentration. Patients were classified according to Child- Pugh score into Child Class A, B and C. [(4)]

Abdominal ultrasonography was done for all patients with special stress on liver echogenicity, presence of any hepatic focal lesions, portal vein diameter, spleen size and amount of ascites. Abdominal Doppler US was done when needed to exclude portal, hepatic vein or IVC thrombosis. Triphasic spiral abdominal CT was done when needed to diagnose hepatocellular carcinoma.

Patients with ascites underwent ascitic fluid paracentesis of 3-4 Liters/day for 3 successive days, and then repeated measurement of the plasma D-dimer level was done in the third post-tapping day (as the reported half-life of plasma D-dimer is 48 hours). [(5)]

Method of blood collection and testing for measuring D-dimer level:

Peripheral blood was collected into tubes containing sodium citrate solution. After centrifugation, the supernatant plasma was removed. Plasma D-dimer was measured by a latex-enhanced, immuno-turbidimetric test using a commercially available kit (D-dimer PLUS, Dade Behring, Marburg, Germany).The D-dimer concentration was expressed in mg/L with a normal value below 0.5 mg/L.[(6)]

Statistical Analysis:

Collected data were analyzed using SPSS (version 16) statistical software package under Windows XP operating system. Qualitative data were presented as frequency (number and %). Chi-square test was used for comparison of qualitative data. Quantitative data were presented as mean ± standard deviation (x ± SD). Paired t-test was used to compare parametric quantitative variables in the same group and Unpaired t-test was used to compare parametric quantitative variables in different groups. Spearman Correlation Coefficient (r) test was used to rank different variables against each other either positive or inverse. Receiver Operating Characteristic (ROC) curve was used to determine the best cut off values of plasma D-dimer for detection of HCC with detection of sensitivity and specificity at this cutoff value. p value <0.05 and <0.01 was considered significant and highly significant, respectively.

Results:

Demographic and clinical data of the studied patients:

A total of hundred patients were included in this study and they were divided into two groups; group A: 50 chronic liver disease patients with ascites and included 30 males (60%) and 20 females (40%) with a male to female ratio 1.5:1. Their ages ranged from 39 to 73 years with a mean age of 54.3±8.6 years. Group B consisted of 50 chronic liver disease patients without ascites; 24 males (48 %) and 26 females (52%) with a male to female ratio 1:1.1. Their ages ranged from 37 to 72 years with a mean age of 54.4±10.6 years. Comparison between both groups revealed no statistically significant differences as regards age and sex ($p>0.05$).

Child classification of studied patients:

According to Child Pugh classification, 20 patients (40%) were Child B and 30 patients (60%) were Child C in group A. In Group B, 26 patients (52%) were Child A and 24 patients (48%) were Child B. Thus, most of Group A patients were Child C and most of Group B patients were Child A with highly statistically significant difference between both groups ($p<0.001$).

Etiology of chronic liver disease in studied patients:

HCV was detected in 44 patients (88%) of Group A in comparison to 32 patients (64%) of Group B, mixed Bilharziasis and HCV infection in 4 patients (8%) of Group A in comparison to 2 patient (4%) of Group B, HBV in two patients (4%) of Group A in comparison to fourteen patients (28%) of Group B and HCV-HBV co-infection in none of Group A patients in comparison to two patients (4%) of Group B. There was highly statistically significant difference between the studied groups ($p<0.001$).

D-dimer level in studied patients:

High plasma D-dimer level (above the normal range) was detected in 70 out of the studied 100 patients (70%). In Group A before paracentesis, all the 50 patients (100%) had high plasma D-dimer level (above the normal range) with mean value of 3.3 ± 2 mg/L. While after paracentesis, plasma D-dimer level returned to normal in 30 patients (60%), decreased to the high normal level in 10 patients (20%) and decreased but remained high above normal level in 10 patients (20%) happened to have HCC. In Group B, the mean plasma D-dimer level was 1.5 ± 1 mg/L and 20/50 patients (40%) who had HCC, their plasma D-dimer level was high (above the normal range).

In Group A, the mean level of plasma D-dimer before ascitic fluid paracentesis was 3.3 ± 2 mg/L and the mean level after paracentesis was 1 ± 1.2 mg/L. Thus, the mean level of plasma D-dimer decreased after paracentesis with 70% percentage of change with highly statistically significant difference.

On comparing Group A before and after paracentesis with Group B, the mean level of plasma D-dimer in Group A before paracentesis (3.3 ± 2 mg/L) was statistically significantly higher than in Group B (1.5 ± 1 mg/L) $p<0.05$, but after paracentesis in Group A, the mean level of plasma D-dimer decreased to 1 ± 1.2 mg/L with no significant difference between both groups $p>0.05$ (Table 1).

D-dimer level in patients with HCC :

Hepatocellular carcinoma (HCC) was detected in 10 patients (20%) of group A and 20 patients (40%) of group B and revealed statistically significant difference between both groups ($p<0.05$).

A highly statistical significant difference was detected on comparing mean plasma D-dimer level in HCC (4.9 ± 1.7) versus non HCC (0.5 ± 0.1) among all the studied 100 patients ($P<0.001$). Regarding the comparison between mean plasma D-dimer level in HCC patients (3.2 ± 1.5) versus non HCC patients (0.5 ± 0.1) among Group A (after ascitic fluid paracentesis), there was highly statistical significant difference between both groups ($p<0.001$). Also, comparison between mean plasma D-dimer levels in HCC patients (5.7 ± 1) versus non HCC patients (0.4 ± 0.1) among Group B showed highly significant statistical difference between both groups ($p<0.001$) (Table 2).

Correlation between plasma D-dimer versus other variables among Group A before and after ascitic fluid paracentesis and among Group B.

In Group A, before paracentesis, plasma D-dimer level was significantly positively correlated with age, Child score and alpha fetoprotein (AFP), but after paracentesis, it was highly significantly positively correlated with AFP only.In Group B, plasma D-dimer level was highly significantly positively correlated with age, Child score and AFP (Table 3).

ROC curve determined that plasma D-dimer of 3.2 was the cutoff value for detection of HCC with 80% sensitivity and 82.9% specificity, showing that plasma D-dimer level is considered a better negative than positive test with higher specificity than sensitivity (Figure 1).

Discussion:

Hemostasis is a dynamic process resulting from the balance between procoagulant and anticoagulant factors. [3] The liver is an important organ that synthesizes coagulation factors, (except the von Willebrand factor); fibrinolytic system proteins, (except the tissue plasminogen factor); and the urokinase-type plasminogen activator; as well as coagulation and fibrinolysis inhibitors. [7] In patients with hepatic parenchymal disease, the loss of functional parenchyma results in decreased synthesis of both coagulation factors and natural anticoagulant proteins. [8]

The deficiency in coagulation factors, platelet dysfunction, thrombocytopenia, dysfibrinogenemia and increased fibrinolysis in patients with liver disease generally present with increased bleeding diathesis [9]; conversely, decreases in antithrombin and other natural anticoagulants increase the risk of thrombosis. Some authors argue that since both coagulation factors as well as antithrombin and natural coagulation inhibitor factors are equally decreased, coagulation is not observed in patients with liver disease. [10] ***Tripodi et al.*** [11] suggested that bleeding is mainly due to the presence of hemodynamic alterations and that conventional coagulation tests are unlikely to reflect the coagulation status of cirrhotic patients.

The incidence of hyperfibrinolysis in patients with liver cirrhosis varies from 19% to 95% and may contribute to serious bleeding complications. [12, 13] Fibrinogen is low whereas plasminogen, alpha-2-antiplasmin and fibrin degradation products are high. In previous study[9] done on patients with acute and chronic viral hepatitis, cirrhosis and HCC, the fibrinogen levels were within normal limits and fibrin degradation products were increased, whereas in another study[7], the fibrinogen level was low, and the fibrinogen molecule was found to contain supernormal sialic acid levels with resultant functional impairment.

More recently Dong et al. [14] showed that D-dimer, a marker of fibrin degradation products, was found to be increased with increasing severity of hepatocyte damage. Since D-dimer was the only parameter that differed significantly between Child A

and Child B patients, D-dimer levels may be considered as an important sign of decompensation in cirrhotic patients.

The finding of high D-dimer plasma concentration in patients with liver cirrhosis decompensated by ascites suggests a major role of ascites in pathogenesis of hyperfibrinolytic state associated with liver failure. [3] High D-dimer levels were also found in patients with HCC without ascites. [15]

In this study, plasma D-dimer levels were above the normal range in 70/100 patients (70%). This agrees with ***Agarwal et al.*** [3] and ***Spadaro et al.*** [16] who reported high D-dimer levels in 63% and 64% of patients with liver cirrhosis respectively. This percentage is higher than that reported in another study[17] where high D-dimer levels were found only in 17% of their chronic liver disease patients. This is because 11 out of their studied 86 patients were not cirrhotic and the number of ascitic patients was not specified in their study.

In the current study, high D-dimer values (above normal level) were more frequent in Group A (100% of patients) than in Group B (40% of patients). In group A before ascitic fluid paracentesis, the mean plasma D-dimer level was (3.3±2 mg/dL) compared to Group B (1.5±1 mg/dL) with statistically significant difference between both groups. This finding is consistent with that of ***Agarwal et al.*** [3] who reported increased plasma D-dimer values in 93% and 33% of patients with and without ascites respectively. Moreover, ***Spadaro et al.*** [16] found high D-dimer levels in 81% of patients with ascites and in 39% of patients without ascites. Other study done by Violi et al. [18] showed that elevated levels of D-dimers were found in up to 93%–100% of patients with advanced stage of cirrhosis with complications such as ascites. ***Gando et al.*** [19] stated that hemostatic molecular markers such as thrombin-antithrombin complex (TAT), plasmin–plasmin-inhibitor complex (PPIC), and D-dimers are frequently elevated in patients with cirrhosis.

In group A before ascitic fluid paracentesis, the plasma D-dimer mean level was (3.3±2 mg/dL) and after paracentesis, the mean level was (1±1.2 mg/dL), that means plasma D-dimer decreased after paracentesis with 70% percentage of change with highly statistically significant difference. In patients of Group A after ascitic fluid paracentesis, the mean D-dimer values returned to normal range in 30 patients(60%), decreased to high normal level in10 patients(20%) and decreased but remained high(above normal level) in10 patients(20%) (HCC was discovered in them). In these patients, D-dimer values after paracentesis of ascites were not significantly different from those found in patients without ascites. Also, in patients without ascites(Group B), high D-dimer levels were associated with presence of HCC(in 20/50 patients; 40%).

These findings agree with ***Spadaro et al.*** [16] who reported that after tapping of ascites, circulating D-dimers decreased significantly in all patients, returning to normal in half of them. Also, mean D-dimer levels in ascitic patients after paracentesis were not significantly different from those in patients who entered the study without ascites. Therefore, our data confirm the association between circulating high D-dimer levels and the presence of ascites found in cirrhotic patients. These findings are consistent with that of ***Agarwal et al.*** [3] who stated the major role of ascites in pathogenesis of hyperfibrinolytic state associated with liver failure.

The underlying mechanism for high D-dimer plasma levels in cirrhotic patients with ascites remains to be clarified. Some authors [3] suggest the exchange of some coagulation and fibrinolytic proteins between plasma and ascitic fluid. ***Violi et al.*** [18] proposed that hyperfibrinolysis in cirrhotic patients might represent a state of low grade disseminated intravascular coagulation secondary to the passage of gut absorbed bacterial material into the systemic circulation.

High mean levels of D-dimer were detected in patients with HCC in both groups in this study and these levels remained high after paracentesis in patients with HCC in Group A. These findings are in agreement with ***Spadaro and his coworkers*** [16] study who found close association between presence of HCC and high D-dimer values. Another study done by ***Kim*** [15] reported high D-dimer levels in HCC patients without ascites.

HCC is often associated with thrombotic invasion of portal or hepatic veins. Although we excluded from our study all patients with features of thrombosis, we cannot rule out the presence of microvascular invasion in patients with HCC. ***Kim et al.*** [20] also found increased circulating D-dimers in patients with HCC, even in absence of tumor thrombosis in a major branch of the portal or the hepatic vein.

In the current study, there was highly significantly positive correlation between plasma D-dimer level and Child score of the patient. This is consistent with that of ***Primignani et al.*** [21] who found that D-dimer level was significantly more elevated in parallel with the worsening of the Child-Pugh class.

Conclusion: High D-dimer is associated either with presence of ascites or HCC. In patients with liver cirrhosis, high D-dimer levels in absence of ascites require more careful monitoring for HCC with a cutoff value of 3.2 mg/L for detection of HCC.

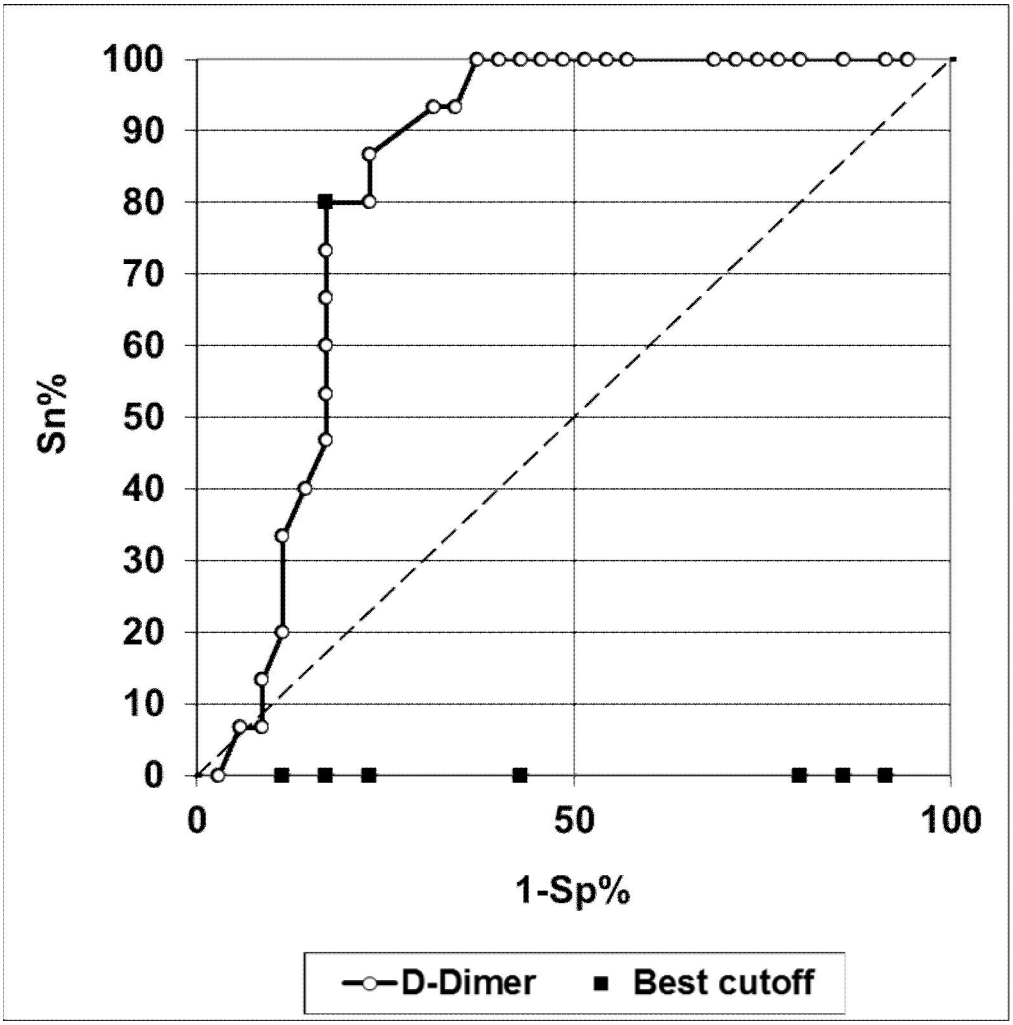

Fig. (1): ROC curve analysis showing the diagnostic performance of D-Dimer for discriminating patients with HCC from those without

Table (1): Comparison between plasma D-dimer level in Group A before and after ascitic fluid paracentesis *versus* Group B:

Parameters	Group A n=50	Group B n=50	P
Before paracentesis (for Group A)	3.3 ± 2	1.5 ± 1	**P<0.05 (S)**
After paracentesis (for Group A)	1 ± 1.2	1.5 ± 1	**P>0.05 (NS)**

Table (2): Comparison between plasma D-dimer level in HCC *versus* Non HCC patients among all patients, Group A (after ascitic fluid paracentesis) and Group B

parameters	HCC	Non HCC	P value
All patients	(n=30) 4.9 ± 1.7	(n=70) 0.5 ± 0.1	**P<0.001 (HS)**
Group A (after ascitic fluid paracentesis)	(n=10) 3.2 ± 1.5	(n=40) 0.5 ± 0.1	**P<0.001 (HS)**
Group B	(n=20) 5.7 ± 1	(n=30) 0.4 ± 0.1	**P<0.001 (HS)**

(N.B. In patients with ascites, the mean plasma D-dimer values were calculated from those measured after paracentesis).

Table (3) Correlation between plasma D-dimer *versus* other variables among Group A before and after ascitic fluid paracentesis and Group B:

Variables	Group A Before paracentesis		Group A After paracentesis		Group B	
	r	P	r	P	r	P
Age	0.43	**<0.05 (S)**	0.06	**>0.05 (NS)**	0.49	**<0.05 (S)**
Child score	0.69	**<0.01 (HS)**	0.11	**>0.05 (NS)**	0.79	**<0.01 (HS)**
AFP	0.47	**<0.05 (S)**	0.75	**<0.01 (HS)**	0.90	**<0.01 (HS)**