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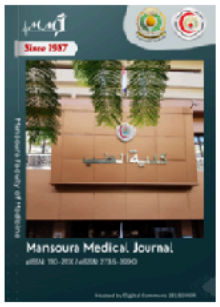

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COAGULATION PROFILE IN CHRONIC LIVER DISEASES

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COAGULATION PROFILE IN CHRONIC LIVER DISEASES

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ABSTRACT

Liver has many hemostatic functions, which include synthesis of most coagulation factors and inhibitors, as well as fibrinolytic factors, except Von Willebrand factor. Also, macrophages in the liver clear many of the activated coagulation, fibrinolytic factors and hemostatic activation complexes. Therefore, it is not surprising that liver diseases result in a complex pattern of defects in hemostatic functions in the form of reduced synthesis of coagulation factors and inhibitors, synthesis of abnormal clotting factors, abnormalities of fibrinolytic activity, disseminated intravascular coagulation and platelets defects.

This study was designed to evaluate the changes in international normalised ratio (INR), activated partial thromboplastin time (APTT), fibrinogen, D-dimer levels, and protein-C antigen concentration in chronic liver diseases patients, and to find out if there is a possible link between these parameters and the pathogenesis of the disease or not. The current study included 55 hepatic patients and 25 age and sex matched healthy controls. The patients divided into two groups, chronic hepatitis C and Post HCV cirrhosis. Plasma fibrinogen level was quantitatively measured by the clotting method of Clauss. Plasma protein C antigen concentration was measured by ELISA kit. D-dimer level was determined by latex agglutination method. INR and APTT were measured by manual coagulation method.

Fibrinogen level and protein C antigen concentration were significantly decreased in patients groups when compared with control ($p<0.001$). D-dimer level, INR as well as APTT were increased in post HCV cirrhotic patients in comparison to the control ($p<0.001$).

The present findings indicate that the prolonged APTT, the increased INR and D-dimer levels, together with decreased fibrinogen level and protein C concentration correlated with the extent and severity of the chronic liver diseases. Therefore, evaluation of these parameters may represent an additional prognostic predictor in such hepatic events and a potential target for early therapeutic interventions.

INTRODUCTION

Liver plays a central role in hemostasis because of its various functions in coagulation. It synthesizes the majority of coagulation and fibrinolytic factors as well as their inhibitors. Furthermore, post-translational modifications of hemostatic factors take place in the hepatocytes e.g. γ-carboxylation of coagulation factors II, VII, IX and X, glycosylation, β- hydroxylation and sialylation. In addition, the liver has a clearance function for activated hemostatic factors and their degradation products [1]. Prothrombin time (PT) measures the time required for a fibrin clot to form when the extrinsic pathway for coagulation is initiated [2]. Its values are useful in the evaluation of patients with deficiencies in the extrinsic pathway of the coagulation cascade [3]. The World Health Organization has adopted a standard thromboplastin reagent and reporting method known as the international normalised ratio (INR) [4].

The intrinsic pathway of the coagulation cascade which is composed of factors V, VIII, IX, X, XI, XII, prothrombin and fibrinogen is assessed by the activated partial thromboplastin time (APTT) [5]. The protein C system exerts its anticoagulant effect by regulating the activities of FVIIIa and FVa, the cofactors in the tenase and prothrombinase complexes respectively. The inhibition of FVIIIa and FVa mediated by the protein C system provides a highly efficient and specific regulation of blood coagula-

tion [6]. Under normal physiological conditions there is a balance of the two opposing processes coagulation and fibrinolysis [7]. D-dimer, a fibrin clot degradation product is released into circulation by the fibrinolytic enzyme plasmin [8]. D-dimer is usually detectable 1 h after thrombus formation and has a circulation half life of 4-6 h. Therefore, its measurement could be used as an initial screening test in patients with clinically suspected thrombosis, as its high negative predictive value rules out ongoing thrombotic complications [9].

The aim of this study was to evaluate the changes in international normalised ratio (INR), activated partial thromboplastin time (APTT), fibrinogen, D-dimer levels, and protein-C antigen concentration in patients with chronic liver diseases, and to find out if there is a possible link between these parameters and the pathogenesis of the disease or not.

SUBJECT AND METHOD

Fifty five hepatic patients were included in the current study. They were selected from patients attending Gastrointestinal Surgery Centre Mansoura University Hospital. They were divided into two groups: twenty five patients with chronic hepatitis C and thirty patients with post HCV cirrhosis. Twenty five healthy subjects with age and sex matched were studied as control. Liver cirrhosis and chronic hepatitis was diagnosed on the basis of clinical evaluation, laboratory, radiological investigation and liver biopsy when available. Qualitative and quantitative detection of serum HCV RNA was done by PCR. Serologic assay for serum antibodies to HCV was performed using third-generation HCV enzyme-linked immunosorbent assay (ELISA 3) (Ortho Diagnosis Systems, Raritan, NJ) [10].

Five ml fasting blood samples were collected in plastic tube containing 0.5 ml of Trisodium citrate (3.2 mg/dl), centrifuged at 3000 rpm for 10 minutes. The separated plasma samples were divided into 3 aliquots: One aliquot was used immediately for measurement of PT, APTT and Fibrinogen concentration. The rest of aliquots were stored at -70°C until assay of D-dimer and protein C. Plasma fibrinogen level was quantitatively measured by the clotting method of

Clauss [11] (Diagnostica Stago, France). Plasma protein C antigen concentration was measured by ELISA kit from (REAADS, Corgenix , U.S.A.) [12]. D-dimer level was determined by latex agglutination method from (ABBOTT murex, U.K.) [13]. INR and APTT were measured by manual coagulation methods [14] and [15] respectively.

STATISTICAL ANALYSIS

The data were expressed as mean \standard deviation for patients and control separately. The statistical differences were analyzed using t-student test for comparison between two groups. p values were expressed and considered significant if <0.05. One way variance analysis (ANOVA test) was done to detect significance among groups. Spearman rank correlation coefficient was done to study the relations between variables. The statistical analysis was performed using SPSS for windows release 13.00 (2004) SPSS Inc.

RESULTS

Table (1) showed that there was a significant prolongation in APTT in post HCV cirrhotic patients when compared with control as well as when compared with chronic hepatitis C patients ($p<0.001$) while there was insignificant difference between chronic hepatitis C patients and control group ($p= 0.16$). Also, there was a significant increase of INR in patients with post HCV cirrhosis when compared to both control and patients with chronic hepatitis C ($p<0.001$).

Table (2) showed that there was a significant decrease in fibrinogen level in post HCV cirrhotic than in control and in patients with chronic hepatitis C ($p<0.001$ and $p=0.02$) respectively. Also, there was significant decrease in protein C concentration in chronic hepatitis C ($p=0.0002$) as well as in post HCV cirrhotic patients ($p<0.001$) when compared to control group. However, there was significant increase in D- dimer concentration in post HCV cirrhotic patients when compared to control and when compared to patients with chronic hepatitis C ($p<0.001$).

Table (3) showed significant positive correlation between APTT and each of INR ($r=0.63$, $p<0.001$), and D-dimer level ($r=0.45$, $p<0.001$).

While there was significant negative correlation between APTT and fibrinogen level (r=-0.31, p<0.001) fig. (1) as well as protein C concentration (r=-0.53, p<0.001). However, there was significant positive correlation between INR and D-dimer (r=0.48, p<0.001) and significant negative correlation between INR and each of fibrinogen level (r=-0.31, p<0.001) and protein C concentration (r=-0.58, p<0.001). Also, protein C concentration correlate positively with fibrinogen (r=0.3, p<0.001) fig. (2), while, it correlates negatively with D-dimer (r=-0.52, p<0.001). There was significant negative correlation between fibrinogen and D-dimmer levels (r=-0.22, p<0.01) fig. (3).

Table (1): APTT and INR in studied groups:

Parameters	Control n=25	Chronic hepatitis C n=25	Post HCV cirrhosis n=30	p
APTT (sec.)	35.20 ±1.19	35.72 ± 1.43	52.47 ± 10.95	p_1= 0.16 p_2< 0.001 p_3 < 0.001
INR	1.01 ± 0.03	1±0.05	1.74±0.64	p_1= 0.62 p_2<0.001 p_3<0.001

p_1=significance between control and chronic hepatitis C groups
p_2=significance between control and post HCV cirrhosis groups
p_3=significance between chronic hepatitis C and post HCV cirrhosis groups

Table (2): Plasma levels of fibrinogen, D-dimer and protein C concentration in studied groups:

Parameters	Control n=25	Chronic hepatitis C n=25	Post HCV cirrhosis n=30	p
Fibrinogen (mg/dl)	343.36±41.75	317.72±56.52	284.57±52.56	p_1=0.07 p_2<0.001 p_3=0.02
Protein C (%)	110.34 ±17.1	79.55 ±20.60	38.66 ±13.31	p_1=0.0002 p_2<0.001 p_3<0.001
D-dimer (µg/ml)	0.41 ± 0.08	1.39 ± 1.17	10.68 ± 10.16	p_1=0.19 p_2<0.001 p_3<0.001

p_1=significance between control and chronic hepatitis C groups
p_2=significance between control and post HCV cirrhosis groups
p_3=significance between chronic hepatitis C and post HCV cirrhosis groups

Table (3): Correlation between the studied variables:

Parameters		APTT (sec.)	INR	Protein C (%)	Fibrinogen (mg/dl)
INR	r p	0.63 <0.001			
Protein C (%)	r p	-0.53 <0.001	-0.58 <0.001		
Fibrinogen (mg/dl)	r p	-0.31 <0.01	-0.31 <0.01	0.3 <0.01	
D-Dimer (µg/ml)	r p	0.45 <0.001	0.48 <0.001	-0.52 <0.001	-0.22 <0.01

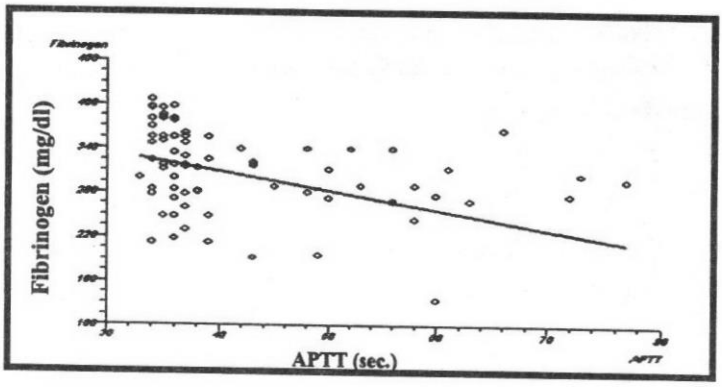

Fig. (1): Correlation between fibrinogen and APTT

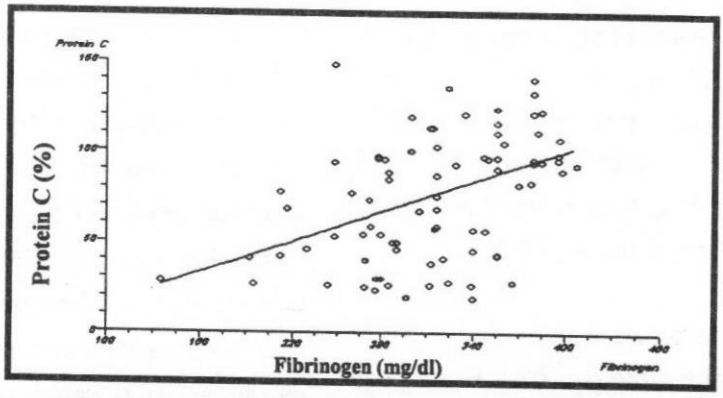

Fig. (2): Correlation between fibrinogen and protein C

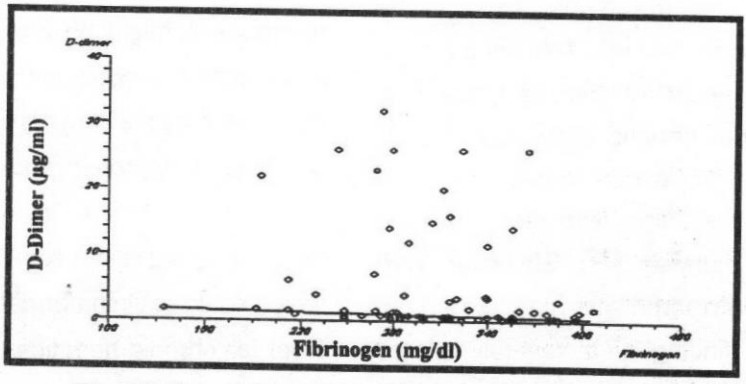

Fig. (3) Correlation between fibrinogen and D-dimer

DISCUSSION

Chronic active hepatitis and the consequent cirrhosis are severe hepatic parenchymal diseases with potentially life-threatening complications. The acute and chronic inflammation in the liver is associated with a bleeding diathesis due to deficiency in the synthesis of coagulation factors as well as a procoagulant state due to the defects in the synthesis of anticoagulant factors by the liver. The levels of some coagulation, fibrinolytic factors and natural anticoagulant proteins synthesized in the liver are valuable in diagnosis and treatment as they reflect the degree of hepatocyte damage [16].

In the present study there was a significant increase of INR in post HCV cirrhotic group which in accordance with the results reported by Gursoy et al. [16]. This could be a consequence of the destruction of functional hepatic mass and impairment in liver synthetic capacity of coagulation factors involved in the extrinsic pathway [17]. However, FVII gene polymorphisms may explain the clinical finding of a severely altered PT in cirrhotic patients who is otherwise in a good functional liver class, or by converse of a normal PT in patients with impaired liver function [18].

In the current study there was significant prolongation of APTT in post HCV cirrhotic patients that could be attributed to a defective hepatic synthesis of coagulation factors involved in the intrinsic coagulation pathway [19].

Also, there was significant decrease of fibrinogen level in post HCV cirrhotic group that in agreement with [16] who reported impaired hepatic synthetic function of fibrinogen. Moreover, this low level could be explained by exchange of fibrinogen into ascitic fluid [20], loss during massive haemorrhage and destruction by enhanced fibrinolytic activity [21]. Furthermore, it might be attributed to a consumption coagulopathy [22]. However, liver has a large synthetic capacity for this protein and can maintain normal blood levels till the advanced stages of liver disease [23] which could explain normal fibrinogen level for chronic hepatitis C group in the current study.

On the other hand results of the present study are controversial with Grimaudo et al. [3] who reported normal fibrinogen level and with Arif et al. [24] who reported elevated fibrinogen level in cirrhosis. These disagreements could be related to different degree of hepatocyte damage.

In the present study there was significant decrease of the protein-C concentration in post HCV cirrhotic as well as in chronic hepatitis C groups as that reported by Gursoy et al. [16] and Al-Ghumlas et al. [25]. They demonstrated that hepatocyte synthetic pathways for protein C are disturbed in chronic hepatitis. Moreover, this low concentration might be related to a concomitant vitamin K deficiency frequently associated with liver disease [20].

However, consumption due to low-grade disseminated intravascular coagulation in advanced liver disease is another possibility [3].

D-dimer is an antigenic determinant of fibrin, which, at the D-region, combines with factor XIIIa, and persists as an X-oligopolymer, as well as with other fibrin degradation products. However, it does not exist with profibrin breakdown products, and is a specific indicator of synthesis and breakdown of fibrin in the system [9].

The current study revealed a significant increase of D-dimer levels in post HCV cirrhotic group which in agreement with the result reported by Gursoy et al. [16]. Increased D-dimer level could be attributed to the hyperactive state of the coagulation pathway and hyperfibrinolysis [26]. In liver cirrhosis hyperfibrinolysis occurs as a result of deficiency of circulating thrombin activable fibrinolysis inhibitor and α 2-antiplasmin. Also it may be due to impaired thrombin generation [27].

However, Agarwal et al. [28] have been found elevated levels of D-dimer and low fibrinogen in ascitic fluid of cirrhotic patients, therefore, reabsorption of ascetic fluid into systemic circulation might contribute to high D-dimer level.

On the other hand there was a significant decrease of protein C concentration together with normal APTT, fi-

brinogen, D-dimer level and INR in chronic hepatitis C patients when compared with healthy controls as reported by [25].

There was significant correlation between fibrinogen level and protein C concentration in the patient groups this in agreement with [25]. Also, there was significant correlation between fibrinogen level and D-dimer. This correlation may indicate consumption of fibrinogen as a result of activation of coagulation system with subsequent activation of thrombin [16].

In conclusion our findings indicate that the prolonged APTT, the increased INR and D-dimer level, together with decreased fibrinogen level and protein C concentration correlated with the severity of the chronic liver diseases. Protein C level may be considered as an early biomarker and a highly sensitive indicator of hepatocellular damage when compared with other hemostatic parameters including INR. Therefore, evaluation of these parameters may represent an early predictive and prognostic indicator in such hepatic events and a potential target for early therapeutic interventions.

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"دلالات التخثر فى أمراض الكبد المزمنة

طارق أحمد حافظ * - محمد عبد الوهاب**

هبه كمال مرسى* - بلال محمد عوض*

قسمى الكيمياء الحيويه الطبيه وجراحة الجهاز الهضمى

هناك توازن بين الأوعية الدموية و صفائح الدم و عوامل التخثر و مضادات التخثر لكى تحافظ على سيولة الدم. أى إضطراب فى هذا التوازن يؤدى الى زيادة القابلية للسيولة او التخثر.إن أمراض الكبد و مضاعفاتها تمثل مشكلة من أكبر المشاكل الصحية فى منطقتنا.وإن السبب الرئيسى لأمراض الكبد المزمنة هو الاصابة بالفيروس الكبدى (ج).

إن الكبد هو المكان الرئيسى لتصنيع الكثير من عوامل تخثر الدم و البروتينات المتحكمة فى ذلك و لهذا ليس بغريب أن يحدث إضطراب فى تخثر الدم عند مرضى الكبد.

هدف البحث إلى دراسة التغيرات التى تحدث فى زمن البروثرومبين , زمن الثرومبوبلاستين ، تركيز الفيبرينوجين، دى ديمر, وبروتين جـ . عند المرضى الذين يعانون من أمراض الكبد المزمنة و إمكانية إستخدام هذه التغيرات كمؤشر على حدوث إختلال فى وظائف الكبد. أجريت هذه الدراسة فى قسم الكيمياء الحيوية الطبية بكلية الطب- جامعة المنصورة ، قسمت الحالات التى تم أخذ عينات الدم منها إلى مجموعتين :- مرضى يعانون من الالتهاب الكبدى الفيروسى المزمن (ج). و تشمل ٢٥ مريض ومجموعة مرضى يعانون من التليف الكبدى و تشمل ٣٠ مريض. للمقارنة تم أخذ مجموعة ضابطة من الأصحاء تتكون من ٢٥ شخصا.

تم أخذ ٥ سم٣ دم من كل شخص و تم فصل البلازما وقسمت إلى ثلاثة أجزاء :جزء لقياس زمن البروثرومبين، معدل سيولة الدم وزمن الثرومبوبلاستين و

الفيبرينوجين والباقى حفظ عند درجة -٧٠ م° لحين إستخدامه فى قياس دى ديمرو بروتين (ج).

اوضحت نتائج البحث:

- إنخفاض ذو دلالة إحصائية عالية فى مستوى بروتين جـ. فى مجموعة مرضى الالتهاب الكبدى الفيروسى المزمن (ج) .
- إنخفاض ذو دلالة إحصائية عالية فى مستوى بروتين جـ و الفيبرينوجين فى مجموعة مرضى تليف الكبد.

زيادة ذات دلالة إحصائية عالية فى معدل سيولة الدم , زمن الثرومبوبلاستين, و دى ديمر فى مجموعة مرضى تليف الكبد.

- علاقة إيجابية ذات دلالة إحصائية عالية بين معدل سيولة الدم و زمن الثرومبوبلاستين, و كذلك بين معدل سيولة الدم و دى ديمر.
- علاقة إيجابية ذات دلالة إحصائية عالية بين بروتين جـ و الفيبرينوجين.
- علاقة سلبية ذات دلالة إحصائية عالية بين بروتين جـ و معدل سيولة الدم, و كذلك بين بروتين جـ و كل من زمن الثرومبوبلاستين, ودى ديمر.
- علاقة سلبية ذات دلالة إحصائية عالية بين الفيبرينوجين وكل من معدل سيولة الدم و زمن الثرومبوبلاستين, و دى ديمر.

نستنتج من البحث :

- أن الزيادة فى مستوى معدل سيولة الدم, زمن الثرومبوبلاستين, و دى ديمر, و النقص فى مستوى بروتين جـ و الفيبرينوجين مرتبط بشدة المرض و لذا يمكن أن تستخدم هذه الدلالات كمؤشر على تطور مراحل المرض و درجة خطورته. كما أن هذه الدلالات و درجة التغيير فيها يمكن أن تعتبر مؤشرا جيدا لبدء العلاج.