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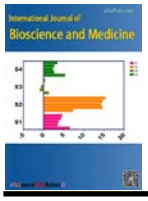

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Value of soluble fibrin as a biomarker for intrahepatic microthrombosis and its sequel acute-on-chronic liver failure in chronic hepatitis C patients

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ABSTRACT

Background: There is a lack in finding the precipitating factor in acute –on chronic liver failure (ACLF)insult in large number of patients and either this factor is hepatic or extra hepatic origin. **Aim of the work** : Our study aiming to evaluate the potential usefulness of a new plasma soluble fibirin polymer (SF) assay for diagnosing the possibility of occurrence of intrahepatic microthrombosis as a cause of ACLF in patients with chronic hepatitis C virus. **Patients & Methods**: The study was carried out in Zagazig University Hospital, internal medicine department in collaboration with microbiology department from February 2015 tell November 2015. 50 patients having chronic hepatitis c virus was enrolled in this study with ACLF developing new onset ascitis in 15 patient encephalopathy in 12 patients, jaundice in12 and elevated INR in 11 patients all having regular follow-up in hepatology clinic in Zagazig university Hospital with stable clinical course in the previous three months. Control subject was classified as normal subject 20 and 30 patients with compensated chronic hepatitis C virus infection. All patients and control groups were subjected to full history, complete clinical examination and laboratory tests including CBC, INR, serum albumin, serum bilirubin, liver enzymes, ascitic fluid examination and culture, blood culture, alpha-fetoprotein, d D-dimer, thrombin generation and soluble fibrin polymer., abdominal ultrasound, Doppler ultrasound for portal vein. **Results:** Our results showed significant difference between patients group and both control groups regarding SF and D-dimer, also there were significant differences in patients group and other groups regarding ALT, total bilirubin especially direct bilirubin.There were marked reduction in portal flow mean

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velocity in patients group and other groups and we recorded further reduction in the portal flow mean velocity in patients group after 2 weeks from the starting time. There is significant positive correlation between SF and D-dimer with ALT, bilirubin, INR, portal flow mean velocity and increasing amount of ascitis and degree of encephalopathy. **Conclusion:** Evaluation the level of soluble fibrin polymer is a useful biomarker to predict ACLF development. Further studies are needed to insure its value and the best modality of treatment in this condition.

Introduction

Acute-on-chronic liver failure (ACLF) is a distinct entity increasingly recognized disease that encompasses an acute deterioration of liver function in patients with chronic liver disease 1. In the year 2002, the London Group proposed a working definition of ACLF: acute deterioration of liver function over a period of 2-4 weeks, usually leading to a serious deterioration in clinical status with jaundice and hepatic encephalopathy and/or Hepatorenal syndrome usually associated with a precipitating event, with a high sequential organ failure syndrome 2.

Although there is no widely accepted ACLF diagnostic criteria, two representative consensus definitions are used. The first was proposed by the Asia-Pacific Association for the study of the liver (APASL) in 2009 3.

Acute hepatic injury manifested as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease ‘.

The second was a working definition of a research consortium of the American Association for the study of liver disease (AASLD) and the European Association for the study of the liver (EASL) 3. proposed; “Acute deterioration of pre-existing, chronic liver disease, usually in connection with a triggering event and associated with a higher mortality rate at 3 months due to multisystem organ failure.

Precipitating events ACLF injury is indicated by hepatic and non-hepatic precipitating events Western experts 3.

Experts also felt that the main precipitating event in acute hepatic failure should be hepatic in origin, however, a considerable proportion of patients with ACLF have no precipitating events. The lack of a specific precipitating factor in about 40% of patients with ACLF is intriguing. This finding puts the impetus on the discovery of new biomarkers that can predict development of ACLF occurring without ‘classic’ precipitating factors 4.

Reduced levels of most procoagulant factors and thrombocytopenia, are the main Haemostatic abnormalities associated with chronic liver disease (CLD). Consequently, this condition, until recently, was considered a prototype acquired coagulopathy. Little attention was paid to the fact that similar to deficiency of procoagulant factors, anticoagulant counterparts (namely protein C [PC] and antithrombin III) are also reduced to the same extent in this setting 5.

Therefore, the probability that a new balance of coagulation could be performed in CLD was ignored for many years. Recently, evidence was provided that the plasma from patients with cirrhosis could create similar or even larger amounts of Thrombin than plasmas from healthy subjects, provided Thrombin generation is measured in the presence of thrombomodulin 6.

Additional reports have linked deficiency of PC, increased expression of FVIII and hyperhomocysteinemia with advanced fibrosis 7.

Early investigators postulated that micro-infarcts resulting from thrombi in branches of the hepatic vein and portal vein near the areas of inflammation caused by ischemia and cell death 8.

Currently there is insufficient data supporting routine use anticoagulation as anti-fibrotic treatment outside of clinical trials. However, it becomes apparent that the modulation of coagulation can be a relevant therapeutic targets for developing new anti-fibrotic factors 9.

Several data suggest that reduced portal flow velocity is an important risk factor for the de-

velopment of thrombosis of Portal vein and that certain genetic prothrombotic factors leading to increased thrombin production can increase the risk, whereas 8% of patients had evidence of hypercoagulability, despite severely reduced levels of coagulation factor level 10.

Interestingly, in a large series of patients, the number of thrombotic events was higher compared with the number of hemorrhagic events, in which these patients do not have a bleeding tendency as suggested by abnormal (PT/INR and APTT) routine laboratory tests, so part of thrombin production profile in these patients suggests hypercoagulability, which is consistent with the clinical observation that thrombotic complications are common in patients with acute liver damage 10.

Moreover, apart from the systemic thrombotic complications, there is evidence of intrahepatic thrombosis in patients with acute on top of chronic liver disease 11.

Animal models of acute liver failure have shown that intrahepatic thrombus formation contributes to the progression of acute liver failure, and have shown that anticoagulant therapy alleviates hepatic injury induced by acetaminophen or Fas ligand 12.

Measurement of D-Dimer (fibrin degradation products) of plasma provides information on the formation of fibrin, followed by fibrinolysis. Currently is used to exclude the diagnosis of venous thromboembolism VTE, due to its excellent negative predictive value (NPV) 13.

However, an increased concentration of D-dimer alone does not confirm the diagnosis of VTE and cannot be used for its positive-predictive value (PPV) since elevated levels of D-dimer can also be observed in patients with malignancy, trauma, ascitis, recent surgery, infection and active bleeding 14.

It was already reported that low soluble fibrin (SF) concentrations in normal plasma are recognized and are found in high concentrations in patients with thrombotic diseases, especially in the early stages15.

SF composition is heterogeneous and depend on the degree of polymerization of fibrin-monomer 16. SF is a marker of thromboembolism with D-Dimer. Calculated accuracy rates also showed that SF specificity for the diagnosis of venous thromboembolism was much greater than the D-Dimer, also the positive predictive value of SF was also greater than D-Dimer in the diagnosis of thromboembolic disease. 17.

Aime of the work:

Our study aiming to evaluate the potential usefulness of a new plasm soluble fibirin polymer (SF) assay for diagnosing the possibility of occurrence of intrahepatic microthrombosis as a cause of ACLF in patients with chronic hepatitis C virus

Subjects&Methods:

This study was carried out in Zagazig University Hospital internal medicine department in collaboration with microbiology department from February 2015 tell November 2015. 50 patients having chronic hepatitis c virus was enrolled in this study with acute on top of chronic liver disease developing new onset ascitis in 15 patients, encephalopathy in 12 patients, jaundice in12 and elevated INR in 11 patients all having regular follow-up in hepatology clinic in Zagazig university Hospital with stable clinical course in the previous three months there were 33 male and 17 female, there ages ranging from 43 to 65 years old.

Patients with systemic infection specially spontaneous bacterial peritonitis, DVT, portal vein thrombosis, osophageal variceal bleeding or rcently injected varices, patients treated previously by anticoagulants.or having hepatoma were excluded from this study

Control subject was classified as normal subject 20 and 30 patients with compensated chronic hepatitis C virus infection.

All patients and control groups were subjected to full history, complete clinical examination and laboratory tests including CBC, INR, serum albumin, serum bilirubin, liver enzymes, ascitic fluid examination and culture, blood culture, alpha-fetoprotein, d D-dimer, thrombin generation and soluble fibrin polymer, also abdominal ultrasound, Doppler ultrasound for portal vein.

Measurement of plasma concentrations of D-dimer and SF:

Plasma D-dimer levels were measured by LPIA-

ACE D-dimer (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) using JIF23 monoclonal antibody (mAb). The JIF23 mAb, which recognizes plasmin-digested N-terminus of the γ-chain on the D region, was used for latex agglutination . SF was also determined by the latex agglutination method using IATRO SF (Mitsubishi Kagaku Iatron, Inc.) containing mAb IF-43, which recognizes a segment of the fibrin Aα chain [(Aα-17–78) residue segment] exposed in the E region of fibrin monomer when the fibrin monomer molecule binds the D region of another fibrin monomer or fibrinogen. The antibody is coated for the SF assay 17.

Statistical Analysis:

The results were presented as mean ± standard deviation (S.D.). Statistical comparisons of individual groups were based on unpaired Student's t-test. The gender ratio was compared with χ2 test. Correlation between variables was done using correlation coefficient "r". P value is considered significant at ≤ 0.05 level, highly significant at ≤ 0.01 and non significant at > 0.05.

Results:

The study included 100 subjects, selected to represent 3 groups: group (I) included 50 patients with ACLF and group (II) included 30 control subjects with compensated liver cirrhosis and group (Ш) included 30 healthy control subjects.

Discussion:

The term of ACLF was first used in 1995 to describe a condition in which two insults to the liver are operating simultaneously, one of them being ongoing and chronic while the other being acute 1. In 2002, the London group proposed a working definition of ACLF: Acute deterioration in liver function over a period of 2-4 weeks, usually associated with a precipitating event, leading to severe deterioration in clinical status with jaundice and hepatic encephalopathy and/or hepatorenal syndrome with a high Sequential Organ Failure Assessment/Acute Physiology and Chronic Health Evaluation II (SOFA/APACHE II) score 2. There have been over 13 different definitions of ACLF to date 2.

Our results showed significant difference between patients group and both control groups regarding SF and D-dimer (table 1) elevated levels of both SF and D-dimer indicate occurrence of thrombosis within the circulatory system, this result is in agreement with Mirshah 13. who suggests that evaluation of plasma SF, in combination with that of D-dimer, represents a potentially useful tool for the early diagnosis of venous thromboembolism, provided that the patients have not been treated previously by anticoagulants.,generally the thrombosis may be disseminated intravascular thrombosis which is excluded by absence of precipitating factors for DIC, no progressive reduction of platelet count, no elevation of APTT during follow up laboratories and absence of schistocytes, all of these can exclude disseminated intravascular coagulopathy and suggest localized thrombosis.

Regarding portal vein thrombosis and DVT were excluded in our study clinically and by Doppler US.

In our study there were significant differences in patients group and other groups regarding ALT , total bilirubin especially direct bilirubin (table2), this indicates that the liver is the main organ affected by this events, these results confirm the results of Edoardo G. Giannini 18. who reported that Liver disease is often reflected by biochemical abnormalities of 1 of 2 different hepatic systems or of liver function . Although tests that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function. A change in serum albumin level or prothrombin time may be associated with a decrease in liver functioning mass. supported by elevated level of INR.

Interestingly there were significant reduction in portal flow mean velocity in patients group and other groups(table 2), also we recorded significant increase in SF, ALT, total bilirubin and INR already with significant reduction in the portal flow mean velocity in patients group after 2 weeks from the admission time (table 3), this means that there is acute increase in the portal pressure, these results are in agreement with Ali Nawaz Khan 19.

who reported that In severe hepatic parenchymal disease, portal venous blood flow is reduced, and a rough correlation is noted between the degree of reduction in portal flow velocity and the severity of hepatic parenchymal disease (providing that studies are performed in strictly fasting patients).

Table 1. Clinical characteristics of patients at admission.

Variable	Mean ± SD	Range
Age (years)	49.1 ± 6.4	(34-61)
BMI (kg/m2)	46.5 ± 6.4	(36-64)
AST (UI/L)	92.2 ±14.7	(64-123)
ALT (UI/L)	84.2 ±12.1	(62-136)
Total bilirubin (mg%)	3.9 ±0.8	(2.8-5.7)
Creatinine (mg%)	0.86 ± 0.15	0.6-1.3))
INR	1.9 ±0.3	(1.6-2.4)
SF	1007.2 ±439.02	(876.8-1236-8)
P -flow	10.7 ± 0.6	(9.2-11.2)
D-dimer	2660.3 ± 162.5	(1987.8-3005.6)
Pts with ascitis	15	
Pts with encephlopathy	12	
Pts with jaundice	42	
Pts with coagulopathy	50	

Table 2 Portal flow and laboratory results of different studied groups.

	Patients	Group1	Group2	F	P
Portal flow	10.7 ± 0.6	12.7 ±0.5	16.5 ±0.59	547.7	.0001
D-dimer	2660.3 ± 62.5	337.6 ±97.7	243.2 ±68.1	41.7	.0001
SF	1007.2 ±439.02	179.9 ±33.7	175.4 ±36.4	70.1	.0001
ALT	84.2 ±12.1	37.9 ±9.3	31.5 ±7.7	208.7	.0001
Bilirubin	3.9 ±0.8	1.5 ±0.2	1.03 ±0.1	192.1	.0001
INR	1.9 ±0.3	1.4 ±0.2	1 ±0.05	90.1	.0001

Table 3 Laboratory results and portal flow changes at admission and after 2 weeks in patients group.

	At admission	After 2 weeks	T	P
Portal flow	10.7 ± 0.6	9.5 ± 0.5	10.62	.0001
D-dimer	2660.3 ± 162.5	2670.4 ± 134.4	.042	.967
SF	1007.2 ±439.02	1157.2 ±494.8	8.43	.0001
ALT	84.2 ±12.1	136.5 ±37.5	8.392	.0001
Bilirubin	3.9 ±0.8	5.2 ±0.9	13.219	.0001
INR	1.9 ±0.3	2.6 ±0.5	9.874	.0001

Table 4 Correlation between SF with bilirubin, INR, Alt and portal flow in the 2nd week.

		Bilirubin	INR	Alt	Portal F. V.
SF		.839**	.748**	.823**	-.642**
		.001	.001	.001	.003
	N	33	33	33	33

The expected explanation of acute reduction of portal flow mean velocity in patients group is due to occurrence of portal tributaries microthrombosis and with absence of portal vein thrombosis excluded by Doppler US this means that thrombosis is localized intrahepatic as microthrombotic lesions in this suggestion may explain the rapid deterioration of liver function tests, in agreement with Early investigators postulated that micro-infarcts resulting from thrombi in branches of the hepatic vein and portal vein near areas of inflammation caused ischaemia and cell death.

Anstee QM suggested that subsequent parenchymal collapse, forming characteristic parenchymal extinction lesions, was eventually replaced by fibrous tissue producing cirrhosis, also thrombotic processes may also be a factor in progression of stable cirrhosis to decompensated hepatic atrophy 20. The association between thrombotic risk factors and advanced staging supports the hypothesis of vascular obstruction for the histological progression of chronic viral hepatitis.14,15 Obliteration of small portal and hepatic veins due to thrombosis and phlebitis has been proposed as an important factor for the progression of chronic liver disease as it results in local hepatocyte death and the development of fibrosis (parenchymal extinction), in fact, in cirrhotic livers, obstruction of small intrahepatic portal and hepatic veins is observed almost invariably 21.Thus changes in the composition of the blood towards a hypercoagulable state in combination with changes in the endothelium of intrahepatic vessels and/or in intrahepatic blood flow, which often develop in chronic liver disease with fibrosis, certainly favour the development of thrombosis in intrahepatic veins. Thus one could hypothesise that patients with chronic viral hepatitis who carry specific genetic or develop more easily specific acquired thrombotic risk factors have a more rapid progression to more severe staging of chronic hepatitis 22.

There is significant positive correlation between SF with ALT, bilirubin, INR and significant negative correlation with portal flow mean velocity (table4) with increasing amount of ascitis and degree of encephalopathy, this correlation is more apparent and more significant in patients after 2 weeks more than at the start of admission this suggesting that continuing lesion and thrombosis is a dynamic and continuing process associated with continuing deterioration in liver functions. Proofing the hypothesis of intrahepatic microthrombosis and its role in deterioration of liver function and progression of cirrhosis may give us the value of using different therapeutic options like LMWH or antithrombin3 studying its effect on protecting the liver from the hypercoagulable state in patients with chronic liver disease.

Conclusion:

Evaluation the level of soluble fibrin polymer is a useful biomarker to predict intrahepatic microthrobosis and its sequel ACLF development. Further studies are needed to insure its value and the best modality of treatment in this condition.

Conflict of Interest

The authors have declared no conflict of interest

Compliance with Ethics Requirements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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