

Significance of Soluble Urokinase Plasminogen Activator Receptor in Liver Cirrhosis

Thesis

submitted for partial fulfillment of Master Degree in Internal Medicine

BY

Amer Mohamed Mostafa Ahmed Hegazy

M.B.,B.Ch., Zagazig University

Supervised By

Prof. Dr. Sayed Mohamed Shalaby

Professor of Internal Medicine

Faculty of Medicine, Ain Shams University

Prof. Dr. Tarek Mohamed Youssef

Professor of Internal Medicine

Director of Gastroenterology Endoscopy Unit

Faculty of Medicine, Ain Shams University

Dr. Mohamed Lotfy Soliman

Lecturer of Internal Medicine

Faculty of Medicine, Ain Shams University

Faculty Of Medicine

Ain Shams University

2013

Acknowledgement

*First and foremost, praises and thanks to **ALLAH** for giving me the grace and privilege to pursue this work and complete it successfully.*

*I wish to express my sincere gratitude and deep appreciation to **Prof. Dr. Sayed Mohamed Shalaby**, Professor of Internal Medicine, Faculty of Medicine Ain Shams University, for his generous guidance and moral support throughout this study. I have been greatly honored to work under his supervision.*

*I also wish to express my utmost gratitude and deep thanks to **Prof. Dr. Tarek Mohamed Youssef**, Professor of Internal Medicine, Director of Gastroenterology Endoscopy Unit, Faculty of Medicine, Ain Shams University, for his continuous guidance, valuable instructions, keen supervision and for offering much of his precious time throughout this study. Without his kind help, the accomplishment of this work would have been much more difficult. It was a great experience and honor for me to work under his supervision.*

*My deep thanks and gratitude are also extended to **Dr. Mohamed Lotfy Soliman**, Lecturer of Internal Medicine, Faculty of Medicine, Ain Shams University, for his continuous support, valuable remarks and keen supervision.*

My sincerest thanks are also extended to all patients included in this study.

My heartfelt gratitude also goes to my dear wife for her caring and support throughout the duration of this study.

Amer Mohamed Hegazy

Contents

Acknowledgement.....	i
Contents.....	ii
List of Tables.....	iii
List of Figures.....	vi
List of Abbreviations.....	viii
Introduction and Aim of the Work.....	1
Review of Literature	
Chapter (1): Liver Cirrhosis.....	4
Chapter (2): Chronic Viral Hepatitis.....	85
Chapter (3): Urokinase Plasminogen Activator Receptor (uPAR)	116
Patients and Methods.....	169
Results.....	177
Discussion.....	195
Summary and Conclusion.....	207
Recommendations.....	210
References.....	211
Arabic Summary	

List of Tables

No.	Title	Page
Table 1	Etiologies of Hepatic Cirrhosis	12
Table 2	Clinical Features of Cirrhosis	16
Table 3	Laboratory Findings in Cirrhosis	22
Table 4	Clinical Laboratory Studies Used in Diagnosing Chronic Liver Disease	26
Table 5	Desired characteristics of noninvasive markers of liver fibrosis	34
Table 6	Differentiation of fibrosis stage F0-1 from F2-4 by serum markers and fibroscan	35
Table 7	Child Pugh Turcotte (CPT) classification	38
Table 8	Model for End-stage Liver Disease Score	39
Table 9	Indications and contraindications for orthotopic liver transplantation	45
Table 10	List of complications of cirrhosis	47
Table 11	Drugs for acute esophageal variceal bleeding	51
Table 12	Treatment of ascites depending on the grade	61
Table 13	Definition and diagnosis of refractory ascites	62
Table 14	New International Ascites Club's diagnostic criteria of hepatorenal syndrome	65
Table 15	Classification of hepatic encephalopathy	71
Table 16	West-Haven criteria for hepatic encephalopathy	72
Table 17	Precipitating factors, tests, and treatment of hepatic encephalopathy	74

Table 18	Antiviral treatment for chronic hepatitis C in adults	98
Table 19	Recommendations for Infected Persons Regarding Prevention of Transmission of HBV to Others	106
Table 20	Components of the PA-system	118
Table 21	Gender distribution in the studied groups and subgroups	178
Table 22	Comparison between the 2 studied groups as regards clinical picture	180
Table 23	Comparison between the different studied groups and subgroups as regards etiology	181
Table 24	Comparison between the different studied groups and subgroups as regards different parameters	183
Table 25	Comparison between the 2 studied groups as regards different parameters	186
Table 26	Comparison between the 2 studied groups as regards mean serum suPAR level	186
Table 27	Comparison between Child class A of group 1 and group 2 as regards serum suPAR level	187
Table 28	Comparison between Child class B of group 1 and group 2 as regards mean serum suPAR level	187
Table 29	Comparison between Child class C of group 1 and group 2 as regards mean serum suPAR level	187
Table 30	Comparison between Child class A and Child class B of group 1 as regards mean serum suPAR level	188
Table 31	Comparison between Child class A and Child class C of group 1 as regards mean serum suPAR level	188
Table 32	Comparison between Child class B and Child class C of group 1 as regards mean serum suPAR level	188

Table 33	correlation between serum suPAR levels and different parameters in the 2 studied groups	189
-----------------	---	------------

List of Figures

No.	Title	Page
Figure 1	Algorithm for the management of complications of cirrhosis	48
Figure 2	Schematic cartoon of the GPI-anchored uPAR	134
Figure 3	Domain structure of human uPAR	140
Figure 4	Consensus sequence and three-finger fold for LU-domains	144
Figure 5	Presence of a uPAR-like gene cluster on human chromosome 19q13 encoding GPI-anchored proteins with multiple LU-domains	147
Figure 6	Crystal structure of uPAR	152
Figure 7	Gender distribution in the 2 studied groups	178
Figure 8	Gender distribution in different Child classes of group 1	179
Figure 9	viral etiology in all studied groups and subgroups	182
Figure 10	mean serum suPAR level in the 2 studied groups	184
Figure 11	mean serum suPAR level in different Child classes of group 1	185
Figure 12	Correlation between serum suPAR level and total bilirubin in group 1 showing a highly significant direct correlation	191
Figure 13	Correlation between serum suPAR level and albumin in group 1 showing a highly significant indirect correlation	192
Figure 14	Correlation between serum suPAR level and PT in group 1 showing a highly significant direct correlation	192

Figure 15	Correlation between serum suPAR level and INR in group 1 showing a highly significant direct correlation	193
Figure 16	Correlation between serum suPAR level and MELD score in group 1 showing a highly significant direct correlation	193
Figure 17	Correlation between serum suPAR level and Child-Turcotte-Pugh score in group 1 showing a highly significant direct correlation	194
Figure 18	Correlation between serum suPAR level and WBCs in group 2 showing a highly significant direct correlation	194

List of Abbreviations

AIDS	Acquired immune deficiency syndrome
ALD	alcoholic liver disease
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANA	antinuclear antibody
ASMA	anti-smooth muscle antibody
AST	aspartate aminotransferase
ATF	Amino-terminal fragment of the A-chain in uPA
CHB	chronic hepatitis B
CHC	chronic hepatitis C
CPT	Child-Pugh Turcotte
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELISA	Enzyme linked immunosorbent assay
EVL	endoscopic variceal ligation
EVO	endoscopic variceal obturation
FGF	Fibroblast growth factor
FPRL	Formyl peptide receptor-like
GGT	gamma glutamyl transpeptidase
GOV	gastroesophageal varices
GPI	Glycosyl-phosphatidyl-inositol
HA	hyaluronic acid
HBeAb	hepatitis B e antibody
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HCC	Hepatocellular Carcinoma
HCV	hepatitis C virus
HE	hepatic encephalopathy
HIV	Human immunodeficiency virus
HSC	hepatic stellate cell
IFN	Interferon

IGV	isolated gastric varices
INR	international normalized ratio
LC	liver cirrhosis
LOLA	L-ornithine-L-aspartate
LPS	Lipopolysaccharide or endotoxin, a component of the gram negative cell membrane
LRP	Low-density lipoprotein receptor-related protein
MCP	Monocyte chemotactic protein
MELD	Model for End-Stage Liver Disease
MHE	minimal hepatic encephalopathy
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
NAFLD	nonalcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
PAI	Plasminogen activator inhibitor
PA-system	Plasminogen activation system
PBC	primary biliary cirrhosis
PCR	polymerase chain reaction
PMN	polymorphonuclear leukocyte
pro-uPA	Proenzyme of uPA (single-chain (sc)-uPA)
PSC	primary sclerosing cholangitis
PT	Prothrombin time
PTT	Partial thromboplastin time
(s)uPAR	Refers to both cell-bound uPAR and suPAR
SBP	spontaneous bacterial peritonitis
serpin	Serine protease inhibitor
suPAR	Soluble urokinase-type plasminogen activator receptor
suPAR(I)	Soluble/free one-domain suPAR
suPAR(I-III)	Soluble/shed three-domain suPAR
suPAR(II-III)	Soluble/shed two-domain suPAR
TGF	Transforming growth factor
TIPS	transjugular intrahepatic portosystemic shunt

TNF	Tumour necrosis factor
tPA	Tissue-type plasminogen activator
uPA	Urokinase-type plasminogen activator (two-chain (tc)-uPA)
uPAR	Urokinase-type plasminogen activator receptor (CD87)
uPAR(I)	Domain I of uPAR
uPAR(II)	Domain II of uPAR
uPAR(III)	Domain III of uPAR
uPAR(I-III)	Cell-bound three-domain uPAR
uPAR(II-III)	Cell-bound two-domain uPAR

Introduction and Aim of the Work

Liver fibrosis and its end-stage sequelae cirrhosis represent a major worldwide health problem (**Bedossa et al., 2003**). Cirrhosis is the most advanced stage of most types of chronic liver disease. It is defined as a diffuse disorganization of normal hepatic structure by extensive fibrosis associated with regenerative nodules. Hepatic fibrosis is potentially reversible if the causative agent is removed. However, advanced cirrhosis leads to major alterations in the hepatic vascular bed and is usually irreversible (**Desmet and Roskams, 2004**).

Inflammation has been identified as the major mechanism that promotes progression of chronic liver diseases, leading to hepatic fibrosis and cirrhosis (**Karlmak et al., 2008**).

At present, liver biopsy is the gold standard to assess the degree of intrahepatic inflammation. In clinical routine, non-invasive, longitudinally measurable biomarkers for local and systemic inflammation would be highly desirable, as they may allow early identification of patients at risk for cirrhosis or at risk for fatal outcome. Nevertheless, currently available laboratory parameters have limitations, because they either reflect hepatic biosynthetic capacity (e.g. albumin, pseudocholinesterase, international

normalized ratio or INR), hepatic cell death [e.g. alanine aminotransferase activity (ALT)], cholestatic damage [e.g. bilirubin, c-glutamyl transpeptidase (GT)] or inflammatory activation restricted to distinct leucocyte subsets [e.g. interleukin (IL)-8 for neutrophils, CXCL10 for T-lymphocytes, monocyte chemoattractant protein (MCP)-1 for monocytes] (**Castera, 2011**).

The urokinase plasminogen activator receptor (uPAR) is expressed on most leucocytes including neutrophils, lymphocytes, monocytes and macrophages, which are crucially involved in the pathogenesis of hepatic inflammation and fibrosis (**Blasi and Carmeliet, 2002 and Smith and Marshall, 2010**). Initially, the receptor has been described to localize the activation of plasminogen to the cell surface after binding of uPA, thus, providing a localized cell surface proteolytic activity (**Behrendt, 2004**). In addition to its functions in the proteolytic cascade, uPAR is a potent regulator of cell adhesion, migration, chemotaxis, proliferation and differentiation through intracellular signaling (**Blasi and Carmeliet, 2002**). In uPAR deficient mice, leucocyte migration is decreased towards sites of inflammation (**May et al., 1998**). Interestingly, uPA- as well as uPAR-knock-out mice were

protected from hepatic fibrosis in experimental liver injury models, possibly because of immunomodulatory effects of uPA in hepatic fibrogenesis (**Higazi et al., 2008**).

In addition to the membrane anchored form, uPAR can be found as a soluble molecule in the serum termed "Soluble urokinase plasminogen activator receptor" (suPAR) (**Blasi and Carmeliet, 2002**).

Functionally, full-length suPAR has been suggested to be a regulator of the uPAR/uPA interaction (**Thunø et al., 2009**). By competing with cell-bound uPAR, suPAR may function as a scavenger for uPA by inhibiting cell-associated plasminogen activation (**Mizukami and Todd, 1998 and Kruger et al., 2000**).

Aim of the work

The aim of this study is to assess the significance of soluble urokinase plasminogen activator receptor (suPAR) in different grades of liver cirrhosis.

Chapter I: Liver Cirrhosis

Definition

The word cirrhosis comes from the Greek word *kirrhos*, which means orange yellow. Laennec gave cirrhosis its name *kirrhos* in 1819 in a brief footnote to his treatise *De l'auscultation mediate* (**Duffin, 1987**).

Cirrhosis is the most advanced stage of most types of chronic liver disease. Cirrhosis is defined as a diffuse disorganization of normal hepatic structure by extensive fibrosis associated with regenerative nodules. Hepatic fibrosis is potentially reversible if the causative agent is removed. However, advanced cirrhosis leads to major alterations in the hepatic vascular bed and is usually irreversible (**Desmet and Roskams, 2004**). Cirrhosis is a progressive and severe clinical condition associated with considerable morbidity and high mortality. It leads to a wide spectrum of characteristic clinical manifestations, mainly attributable to hepatic insufficiency and portal hypertension (**Afdhal, 2004**).

Cirrhosis can remain compensated for many years before the development of a decompensating event (**D'Amico et al., 2006**). In most persons, approximately 80 to 90 percent of the liver parenchyma must be destroyed before liver failure is manifested clinically (**Heidelbaugh and Bruderly, 2006**). Decompensated cirrhosis is marked by the development of any of the following complications: jaundice, variceal hemorrhage, ascites, or encephalopathy (**D'Amico et al., 2006**).