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صدق الله العظيم

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TOWARDS A SCORING SYSTEM DEPENDING ON LABORATORY AND ULTRASOUND FINDINGS FOR THE DETECTION OF HEPATIC FIBROSIS IN CHRONIC HCV INFECTED PATIENTS

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ABSTRACT

Non-invasive markers of liver fibrosis have recently been developed as an alternative to liver biopsy.

The aim of this study to Built a novel scoring system that evaluates all the stages of fibrosis and the grading of inflammation, using serum markers and Doppler ultrasonography, in a trial to give data comparable to liver biopsy in HCV patients.

METHODS: We studied 60 chronic hepatitis C patients using ELISA test to detect hyaluronic acid & RID tests to detect haptoglobin, apolipoprotein A1& α2 macroglobulin & conventional & duplex-Doppler abdominal ultrasonography. Liver fibrosis was staged according to the METAVIR scoring system. **RESULTS:** The study revealed significant positive correlation as regards the age , HAI, HV, liver texture, LT lobe DIA, SV DIA, HAPS, AST, HA, α2macroglobulin, APRI & modified APRI in relation to fibrosis stages. The diagnostic performance of scores determined by AUROCs, discriminating F0F1 versus F2F3, was 0.762 for APRI & 0.740 for Modified APRI, at cut off 0.5 & 4.73 with PPV 0.75 & 0.77, NPV 0.75 & 0.70, sensitivity 91% & 82% & specificity 54% & 64% respectively. The AUROCs were 0.660 for hyaluronic acid & 0.692 for α2macroglobulin at cut off 40 &165.65 with PPV 0.75 & 0.81, NPV 0.54 & 0.64, sensitivity 62% & 74% & specificity 68% & 73% respectively. Using logistic regression, statistical independence was demonstrated for combined SV V_{max} & HA/ SV V_{mean} & HA.

CONCLUSIONS: Current non-invasive tests HA, α2macroglobulin & APRI, modified APRI scores give reliable information on liver fibrosis in chronic hepatitis C patients, especially when used in combination but the use of ultrasonograghy & Doppler indexes is unclear yet & thus need further evaluation.

Key words:

- Fibromarkers
- Alpa2macroglobulin
- Hyaluronic acid
- Doppler ultrasonography
- Hepatitis C
- Fibrosis

INTRODUCTION

Chronic liver diseases are characterized by two fundamental aspects, persistent injury and fibrosis, which are cardinal features for the continuous progression toward cirrhosis and end stage liver failure ***(Torre et al.,* 2008).** Liver fibrosis represents the generic wound healing response to chronic insults regardless of their mechanism. The causes of chronic liver injury and fibrosis have a wide geographic distribution and include chronic viral hepatitis, parasitic disease, inborn errors of metabolism, toxic damage, alcohol and non alcoholic fatty liver disease **(Iredale, 2008).**

Hepatic fibrosis refers to the accumulation of interstitial or 'scar' extracellular matrix (ECM) after either acute or chronic liver injury **(Friedman, 2008).** The excess deposition of (ECM) involves molecular and histological rearrangement of various types of collagens, proteoglycans, structural glycoproteins and hyaluronic acid. It is a hallmark of liver cirrhosis and contributes significantly to the deleterious outcome of chronic liver diseases. The deposition of ECM in the space of Disse, the generation of subendothelial basement membranes, and the strangulation of hepatocytes by a surrounding matrix impair not only the blood flow through the organ, but also the biosynthetic function of hepatocytes and the clearance capability of these and other cells **(Gressner et al., 2007b*).*** Cirrhosis, the end-stage of progressive fibrosis, is characterized by septum formation and rings of scar that surround nodules of hepatocytes. **(Friedman, 2008)**

Following significant progress in understanding its underlying mechanisms, hepatic stellate cells (HSCs) undergo a phenotypic switch induced within the inflammation process by numerous cells and cytokines & results in

increase of the (ECM) **(Torre et al., 2008).** Efforts have focused on the hepatic stellate cell, as they can undergo 'activation' into proliferative and fibrogenic myofibroblast-like cells during liver injury. Stimuli driving stellate cell activation include hepatocellular necrosis due to oxidant stress, apoptosis, and soluble growth factors. Specific lymphocyte subsets can also stimulate fibrogenesis. A cascade of signaling and transcriptional events in stellate cells underlies the fibrogenic response to liver injury, with each step in the cascade being a potential target for antifibrotic therapy. Disease-specific fibrogenic mechanisms have also been uncovered: in hepatitis C, by direct stimulation of stellate cell activation by viral infection. **(Friedman, 2008).**

The composition of extracellular matrix molecules in the fibrotic liver is similar to those of other fibrosing parenchyma, including lung and kidney, and different etiologies of liver disease. Typically fibrosis requires years or decades to become clinically apparent **(Friedman., 2008).** Human studies now indicate that fibrosis and even cirrhosis could be reversible, especially if the underlying disease is eradicated. A key challenge is to establish noninvasive means of assessing fibrosis stage and progression using either serum tests and/or imaging. In addition, endpoints of antifibrotic clinical trials need to be established so that reliable evidence of benefit can be identified **(Friedman, 2008).**

Hepatitis C virus (HCV) infection is an important public health problem because approximately 170 million people are infected worldwide. Chronic liver disease results from persistent infection in the majority of patients infected with the virus **(Snyder et al., 2007)** related mortality and morbidity are the result of fibrosis development leading to cirrhosis, which occurs in 10 to 25% of cases **(Fontaine et al., 2002).**

Current guidelines published by the National Institutes of Health (NIH) recommend treatment for significant fibrosis **(Snyder et al., 2007).** Thus, diagnosis, follow-up and therapeutic monitoring of fibrogenesis is of great clinical importance **(Gressner et al., 2007c).** Until now, the gold standard to assess the stage and progression of any liver disease has been the liver biopsy and consecutive histological evaluation based on various numerical scoring systems (Knodell, Ishak, METAVIR, Scheuer, Desmet and others) leading to grading of necroinflammatory activity and staging of fibrosis **(Gressner et al., 2007c).** Although the use of standardized scoring systems in evaluating biopsies has reduced inter-observer variability, sampling variability still remains problematic **(Snyder et al., 2007).**

This approach is further from being ideal, due to the risks and costs & impractical to diagnose and stage liver diseases affecting large segments of the population or to monitor disease progression or treatment effects **(Bonekamp et al., 2009).** Alternative approaches are strongly looked for. Serum markers of cytolysis have been defined and used since 1950s **(Torre et al., 2008)**.The use of such markers could potentially reduce the number of biopsies, follow the progression of liver disease & distinguish patients with mild from those with significant fibrosis. Ideally, these markers would be liver-specific, reflecting the severity of fibrosis, and would also be easy to calculate **(Snyder et al., 2007).**

The ideal non-invasive test is simple and reproducible, readily available, less expensive than biopsy, and able to predict the full spectrum of fibrosis and reflect changes occurring with therapy. Serum marker panels and indices composed of routine laboratory parameters have been used for

this purpose and their diagnostic accuracy (DA) published **(Bonekamp et al., 2009).**

The serum markers α_2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glutamyl-transpeptidase (GGT), and total bilirubin, ALT, platelet count, aspartate-aminotransferase to platelet ratio index (APRI), hyaluronic acid, have been included in different combinations in several models. FibroTest (FT) and Actitest (AT) which is a simple non-invasive panel of biochemical markers for fibrosis and activity **(Haflon et al., 2005)** can distinguish patients without fibrosis F0-F1 versus patients with fibrosis F2-F3-F4, as an alternative to liver biopsy in patients with chronic hepatitis C **(Halfon et al., 2003)** so could be used to reduce the number of liver biopsies done in patients with chronic HCV infection **(Imbert-Bismut et al., 2001).** However, it must be interpreted with caution due to the possible variations of its different constituents not linked to hepatic fibrosis **(Pariente., 2005).** Studies by Poynard's group have shown that FibroTest provides a linear biochemical evaluation of liver fibrosis **(Afdhal, 2004).** The Forns model accurately predicts the absence of significant fibrosis and might render liver biopsy unnecessary in more than one third of patients with chronic hepatitis C **(Forns et al., 2002).** The Forns model and the APRI model can be very useful for prediction of liver fibrosis, mainly in patients with genotype 1 when are used together **(Gomez et al., 2005).** The model Hepascore provides useful information regarding different fibrosis stages among hepatitis C patients **(Adams et al., 2005).**The "Fibrometer" test has a high diagnostic accuracy for significant fibrosis and provide a quantitative estimation of the amount of fibrosis, which is especially useful in cirrhosis **(Cales et al., 2005).** Among the isolated hepatic blood markers, the prothrombin level and the hyaluronic acid have good specificity but insufficient sensitivity for the diagnosis of severe fibrosis **(Pariente, 2005).**

The major pitfall of all of the current serum fibrosis markers is their lack of ability to differentiate small changes in the state of the extra-cellular matrix **(Afdhal**, **2004).**

It is difficult to justify serial liver biopsies to diagnose and monitor patients with chronic HCV when there are limited options for managing their disease in Egypt, where there is a heavy chronic liver disease burden since diagnosing severe fibrosis or cirrhosis could initiate strategies for treatment of HCV and screening for hepatocellular carcinoma **(Esmat et al., 2007).** ***Bishr et al., (2004)*** revealed that α_2-macroglobulin, haptoglobin, platelet count, APRI, γ-glutamyl-transpeptidase (GGT) are valuable in predicting the presence of significant fibrosis in HCV patients with α_2-macroglobulin, GGT, platelet count, APRI, and AST/ALT are highly significant marker of both hepatic fibrosis and disease activity. ***Esmat et al., (2007)*** revealed that young patients Egyptians with chronic HCV, with low levels of HA are at very low risk of fibrosis & this can limit the number of liver biopsies to those whose clinical findings conflict with the biomarker results.

With improvements in imaging technology and advances in contrast media development, the role of radiologic modalities in the detection, characterization, and monitoring of liver disease has significantly increased during the last decades **(Bonekamp et al., 2009)**.

Ultrasound has a good specificity but its sensitivity is insufficient **(Pariente., 2005).** Doppler ultrasonography is sensitive to hemodynamic alterations resulting from inflammation and fibrosis, and if sonography is the study of choice to follow the progression of hepatitis, it will not be adequate without Doppler imaging as it has high diagnostic accuracy (DA)

in cirrhosis **(Haktanir, et al., 2005).** In Egypt, ***Al-Dhafari et al., (2003)*** concluded that abdominal ultrasongraphy increases the (DA) in patients with compensated chronic liver disease especially the application of duplex-Doppler ultrasonography. There was a significant negative correlation between hepatic artery resistive index (HARI) and HAI. There was a highly significant positive correlation between liver surface and fibrosis stages and a significant negative correlation between portal vein peak velocity (PV V_{max}) and fibrosis stages. Another scoring model including platelet count , matrix metalloproteinase-9 (MMP-9), portal vein diameter, splenic longitudinal axis, ALT, AST and viral load couldn't completely replace liver biopsy but factors may play a major role in forecasting the course of HCV as well in determining the therapeutic approach in each case **(El-Shorbagy et al., 2004).**

Objective of the study

Build a novel scoring system that evaluates all the stages of fibrosis and the grading of inflammation, using serum markers and Doppler ultrasonography, in a trial to give data comparable to liver biopsy in HCV patients.

INDEX

LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ADAMS-13	*A disintegrin & metalloproteinase with thrombospondin* type repeats
ALT	Alanine transaminase
AMA	Antimitochondrial antibody
ANA	Antinuclear antibody
APOA1	apolipoprotein A1
APRI	AST to platelet ratio index
a-SMA	α-smooth muscle actin
AST	Aspartate transaminase
AT	Actitest
AUC	Area under curve
CBC	Complete blood count
CHC	Chronic hepatitis C
CI	Confidant interval
CLDs	Chronic liver diseases
CPT1A	Carnitine palmitoyltransferase 1A
CRS	cirrhosis risk score
CS	Cross section
CT	Computerized tomography
CTGF	connective tissue growth factor
Cyp2E1	Cytochrome P450 2E1
DA	Diagnostic accuracy
DDR2	Discoidin domain receptor 2
DIA	Diameter
ECE	Endothelin -converting enzyme
ECM	Extracellular matrix
EGF	Epidermal growth factor
EHS matrix	Englebreth-Holm-Swarm matrix
ELF	Enhanced liver fibrosis
ELISA	Enzyme linked immunosorbant assay
ET-1	Endothelin-1
FGF	Fibroblast growth factor
FIB4	Age , AST, ALT and platelet
FT	Fibrotest
H & E	Hematoxylin-eosin
HA	Hyaluronic acid

HAI	Histological activity index
HAPS	Hepatic artery peak systole
HARI	Hepatic artery resistive index
Hb	Haemoglobin
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HSCs	Hepatic stellate cells
IGF-1	Insulin -like growth factor type 1
IL	Interleukin
INF γ	Interferon gamma
IV	Intravenous
JI	Jejuno-ileal
Jnk	Jun terminal kinase
kDA	*Kilodalton*
kPa	kilopascals
LANGFR	Low-affinity nerve growth factor receptor
MCP-1	Monocyte chemotactic protein-1
MELD	Model for end-stage liver disease
MHz	Mega hertz
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MT1-MMP	Membrane type 1 matrix metalloproteinase
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-kB	Nuclear factor kB
NGF	Nerve growth factor
NO	Nitric oxide
NPV	Negative predictive value
OB-R_L	Long-form leptin receptor
PAI-1	Plasminogen activator inhibitor-1
PBC	Primary biliary cirrhosis
PBMCs	Peripheral blood mononuclear cells
PC	Prothrombin concentration
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PGA	Prothrombin, gamma glutamyl transferase and apolipoprotein A1

PICP	Procollagen I carboxy terminal peptide
PIIINP	Procollagen III amino terminal peptide
PIIINP	type III procollagen
PPARs	Peroxisomal proliferator activated receptors
PPV	Positive predictive value
PT	Prothrombin time
PV	Portal vein
QTL	quantitative trait locus
RGD	Arg-Gly-Asp
ROC	Receiver operating characteristics
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
SD	Standard deviation
SHASTA	Serum hyaluronic acid, AST, and albumin
SNPs	Single nucleotide polymorphisms
SV	Splenic vein
TGF- β	Transforming growth factor β
Th	T Helper lymphocyte
TIMP	Tissue inhibitor of metalloproteinase
TLR4	Toll like receptor 4
TNFR	Tumor necrosis factor receptor
TNFα	Tumour necrosis factor alpha
TPN	Total parenteral nutrition
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
uPA	Uroplasminogen activator
VEGF	Vascular endothelial cell growth factor
VLDL	Very-low-density lipoprotein
V_{max}	Maximum velocity
V_{mean}	Mean velocity
WBC	White blood cell
γGT	Gamma glutamyl transferase

LIST OF TABLES