Value of serum hyaluronic acid assessment in comparison to other non invasive fibrotic markers for predicting liver fibrosis in chronic hepatitis C patients

By

Sameh El Sayed Abo Raia Abo El Enin

M.B., B.ch; M. Sc. Internal medicine

Thesis
Submitted for partial fulfillment of
MD Degree
In
Internal medicine

Supervisors

Prof. Dr. Hoda Al-Tayeb Nasser
Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Prof. Dr. Rawia Abd Al-Salam Ibrahim Al-Fiky
Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Prof. Dr. Mohammed Abd El-Mabood Mohammed
Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Dr. Amany Talaat kamal
Assistant Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Dr. Sherif Hamed Abou-Gamrah
Assistant Professor of radiodiagnosis
Faculty of Medicine, Ain Shams University

(2010)

Acknowledgement

Thanks God at the beginning and at the end of any work. In the preparation of this work, I have received great help from many sources. I would like to express my feeling and how much I'm grateful to all whom with their time and effort have assisted me.

I would like to express deep appreciation to ***Prof. Dr. Hoda Al-Tayeb Nasser*** Professor of Internal medicine, Faculty of Medicine, Ain Shams University for her continuous instructive education, guidance, encouragement and supervision.

Also, I would like to express my gratefulness to ***Prof. Dr. Rawia Abd Al-Salam Ibrahim Al-Fiky*** Professor of Internal medicine, Faculty of Medicine, Ain Shams University for her supervision and guidance in preparation of this study.

I would like to express my deep thanks to ***Prof. Dr. Mohammad Abd Al-Mabood Mohammed*** Professor of Internal medicine, Faculty of Medicine, Ain Shams University for his continuous great help and encouragement throughout the whole work.

I am also quite grateful to ***Dr. Amany Talaat kamal*** Assistant Professor of Internal medicine, Faculty of Medicine, Ain Shams University for her great help and support.

My great thanks to ***Dr. Sherif Hamed Abou-Gamrah*** Assistant Professor of radiodiagnosis, Faculty of Medicine, Ain Shams University for his help and valuable directions.

I am quite grateful to ***Dr. Abeer Ebrahim Rezk*** lecturer of clinical pathology, Faculty of Medicine, Ain Shams University for here help in samples assessment and results of this work.

My great thanks to ***Prof. Dr. Ahmed M. El Sawy*** professor of Internal medicine, Military academy, my teacher for his kind support and continuous encouragement.

I would like to express my great appreciation to ***Dr. Mie Ali Ali mohammed*** lecturer of histopathology, Faculty of Medicine, Mansoura University, my wife for her continuous help and participation in the results of this study.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A2M	α2-macroglobulin
AGSCP	Annealing genotype-specific capture probes
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AT	ActiTest
AUC	Area under curve
BA	Biliary atresia
BCG	Bromcresol green
CDKs	Cyclin dependent kinases
CKIs	Cyclin dependent kinases inhibitors
Col-IV	Type IV collagen
CP	Child-Pugh
CT	Computed tomography
CTLs	Cytotoxic t lymphocytes
Da	Dalton
DC	Denderitic cells
DNA	Deoxyribonucleic Acid
DPD	2,5-dichlorophenyldiazonium
ECM	Extracellular matrix
EDA	Extra domain A
EIAs	Enzyme immunoassays
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
Fb	Fibroblast
FT	FibroTest
GAG	Glycosaminoglycan
GGT	γ-glutamyl transpeptidase
GGTP	Gammaglutamyl transferase
GlcA	Glucuronic acid
GlcNAc	N-acetylglucosamine
GM-CSF	Granulocyte macrophage colony-stimulating factor
GT	Genotype
GTP	Guanosine-5'-triphosphate
HA	Hyaluronic acid
HABPs	Hyaluronic acid binding proteins
Has	Hyaluronic acid synthase
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
HIS	Hepatitis syndrome

HIV	Human immunodeficiency virous
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
HSCs	Hepatic stellate cells
HS	Highly significant
Hyal	Hyalurnidases
ICAM-1	Intercellular adhesion molecule-1
IDUs	Injection drug users
IFCC	International Federation of Clinical Chemistry
IFN	Interferone
Ig	Immunoglobulin
IGFBP-1	Insulin-like Growth Factor Binding Protein-1
IL	Interleukin
IQR	Interquartile range
IRES	Internal ribosome entry site
Kd	Kilo Dalton
LECs	Liver sinusoidal endothelial cells
LN	Laminin
LYVE-1	Lymphatic vessel endothelial HA receptor-1
MCP-1	Monocyte chemotactic protein-1
MELD	The model for end stage liver disease
MF	Myofibroblast
mg	Milligram
MH-S	Murine alveolar macrophage cell line
MIP	Macrophage inflammatory protein
mL	Milliliter
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
mRNA	Massenger ribonucleic acid
MW	Molecular weight
NAFLD	Nonalcoholic fatty liver disease
NaN3	sodium azide
NASH	Nonalcoholic steatohepatitis
ng	Nanogram
NK	Natural killer
NNIs	Nonnucleoside polymerase inhibitors
NPV	Negative predictive value
NS	Nonstructural protein
NSB	Nonspecific binding
NTPase	N-terminal protease
OA	Osteoarthritis
O.D	Optical density
ORF	Open reading frame

P21	Protein 21 Kilodalton
P53	Protein 53 Kilodalton
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PBC	primary biliary cirrhosis
PEG-IFN	Pegylated interferon
PPV	Positive predictive value
PTR	Proteoglycan tandem repeat
r	Spearman rank correlation coefficient
RHAMM	Receptor for hyaluronan-mediated motility
PIIINP	Procollagen type III
RIA	Radioimmunoassay
RNA	Ribonucleic acid
ROC	Receiver Operator Characteristic
RT-PCR	Real-time polymerase chain reaction
RVR	Rapid virologic response
SEC	Sinusoidal endothelial cells
SHAP	Serum-derived hyaluronan-associated protein
SVR	Sustained virologic response
t1/2	Half-life
TIMPs	Tissue inhibitors of metalloproteinases
TMA	Transcription-mediated amplification
TLR4	Toll like receptor 4
TNF	Tumor necrosis factor
TSG-6	Tumor necrosis factor-stimulated gene 6
ug	Microgram
µL	Microliter
UTR	Untranslated region
VCAM-1	Vascular cell adhesion molecule-1
WHO	Worled health organization

PROTOCOL FORM

(MD thesis)

Internal medicine

==========

Value of serum hyaluronic acid assessment in comparison to other non invasive fibrotic markers for predicting liver fibrosis in chronic hepatitis C patients

Thesis
Submitted for partial fulfillment of
MD Degree
In
Internal medicine
By

Sameh El Sayed Abo Raia Abo El Enin

M.B., B.ch; M. Sc. Internal medicine

Supervisors

Prof. Dr. Hoda Al-Tayeb Nasser
Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Prof. Dr. Rawia Abd Al-Salam Ibrahim Al-Fiky
Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Dr. Mohammad Abd Al-Maabood Mohammad
Assistant Professor of Internal medicine
Faculty of Medicine, Ain Shams University

Dr. Amany Talaat kamal
Lecturer of Internal medicine
Faculty of Medicine, Ain Shams University

Dr. Sherif Hamed Abou-Gamrah
Lecturer of radiodiagnosis
Faculty of Medicine, Ain Shams University

(2006)

Introduction:

The number of patients who are diagnosed with chronic hepatitis C has significantly increased in recent years. This is due to several factors, including better knowledge of the disease by general practitioners and specialists, advances in serologic and virologic testing for hepatitis C virus (HCV), and the existence of more effective therapeutic options **(Forns and Bataller, 2003)**.

Hepatitis C virus–induced liver injury consists of hepatocyte damage, which causes oxidative stress and induces the recruitment of inflammatory cells, mainly lymphocytes **(Schuppan *et al*., 2003)**. Both factors activate hepatic stellate cells, which secrete large amounts of collagen leading to progressive fibrosis. In advanced stages, liver fibrosis is massive and cirrhosis develops. Most of HCV morbidity and mortality is due to the development of liver fibrosis. Therefore, an accurate diagnosis of liver fibrosis is very important in the evaluation of patients with chronic hepatitis C **(Poynard *et al*., 2000)**.

Fibrosis with its endpoint, cirrhosis, is the main complication of chronic hepatitis C. It is a key histologic feature in chronic hepatitis useful for evaluation of severity of the disease, for treatment decisions, and for assessing drug efficacy. To date, liver biopsy remains the gold standard for fibrosis assessment in hepatitis C, but this procedure has several limitations including morbidity and mortality, observer variability, and sampling variation **(Bedossa *et al*., 2003)**.

The risk of developing cirrhosis depends on the stage (degree of fibrosis) and the grade (degree of inflammation and necrosis) observed in the initial liver biopsy **(Halfon *et al*., 2005)**, however, liver core biopsy specimen represents only a very limited

part of the whole liver and fibrosis is a heterogeneously distributed lesion, as pointed out by several pioneer studies **(Gascon-Barri *et al*., 1989)**.

Hyaluronate is a polysaccharide with molecular weight ranging from 4×10^3 to 8×10^6 Daltons. It forms a constituent of extracellular matrix in all connective tissues. It is mainly produced by mesenchymal cells and cleared by hepatic sinusoidal endothelial cells through a high affinity receptor; it has a short half life and increases by age **(Tamaki *et al*., 1996)**.

Alcohol, viruses, auto immune diseases, and inborn errors of metabolism could increase production of hyaluronate by activating hepatic stellate cells and decrease clearance by hepatic sinusoidal capilarization. Sinusoidal capilarization could be associated with shunting of blood which is an additional factor for increase of serum hyaluronate in this condition. It was shown that serum hyaluronate increase in alcoholic liver disease, primary billiary cirrhosis and in patients with hepatitis C. In addition, it could be increased in rheumatoid disease due to overproduction by synovial cells. It also increases in renal failure because of disturbed clearance of low molecular weight hyaluronate by the kidneys **(Montazeri *et al*., 2005)**.

Aim of work:

The aim of this work is to evaluate the diagnostic value of Hyaluronic acid for detection of fibrosis and cirrhosis, in patients with chronic HCV infection and compare its results, as a non invasive marker, with the results of histopathological study.

Subjects and methods:

All cases are collected from Ain Shams university hospitals.

During the period of the study, the studied subjects are classified into two groups:

- Group (1) which consists of 60 patients with already diagnosed chronic HCV infection.
- Group (2) which consists of 20 healthy controls.

Both groups will be subjected to:

1. Full clinical history (excluding alcohol intake) and thorough clinical examination.
2. Routine laboratory assessment including liver (serum transaminases, albumin, bilirubin, prothrombin time) and kidney (blood urea and creatinin) function tests and complete blood picture to exclude any other associated hidden disease
3. Ultrasound evaluation.
4. Liver biopsy (group (1)), after taking consent, with adequate core length and portal tracts.
5. Assessment of hyaluronic acid level by enzyme linked immunosorbent assay (ELISA). All serum samples will be obtained in the day of liver biopsy
6. Assessment of serum procollagen.

Exclusion criteria: Any patient with kidney disease, joint injury, alcohol intake will be excluded from the study.

References

1. **Bedossa P, Darg`ere D, and Paradis V (2003):** Sampling Variability of Liver Fibrosis in Chronic Hepatitis C. Hepatology; 38:1449-1457.

2. **Forns X and Bataller R (2003)**: Can we identify liver fibrosis in HCV-infected patients without a liver biopsy? Current Hepatitis Reports, 2:145–151

3. **Gascon-Barre M, Huet PM, Belgiorno J, Plourde V, Coulombe PA (1989):** Estimation of collagen content of liver specimens. Variation among animals and among hepatic lobes in cirrhotic rats. J Histochem Cytochem; 37:3:377-381.

4. **Halfon P, Bourlière M, Pénaranda G *et al*., (2005):** Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. Comparative Hepatology, 4:6

5. **Montazeri G, Estakhri A, Mohamadnejad M *et al*., (2005):** Serum hyaluronate as a non-invasive marker of hepatic fibrosis and inflammation in HBeAg-negative chronic hepatitis B. BMC Gastroenterology, 5:32.

6. **Poynard T, Ratziu V, Benhamou Y, *et al*., (2000):** Natural history of HCV infection. Baillieres Best Pract Res Clin Gastroenterol, 14:211–228.

7. **Schuppan D, Krebs A, Bauer M, et al., (2003):** Hepatitis C and liver fibrosis. Cell Death Differ, 10(suppl 1):S59–S67.

8. **Tamaki S, Ueno T, Torimura T, Sata M, Tanikawa K, (1996):** Evaluation of hyaluronic acid binding ability of hepatic sinusoidal cells in rat liver with liver cirrhosis. Gastroenterology, 111:1049-1057.

INTRODUCTION AND AIM OF WORK

Introduction

Hepatitis C virus (HCV) is a major public health problem and one of the leading causes of death from liver disease **(strader et al., 2004)**. Being the second most common chronic viral infection in the world with a global prevalence of about 3% (about 180 million people) **(Craxi et al., 2008)**.

Egypt has the highest HCV prevalence in the world (overall prevalence of HCV is 12% among the general population, reaches 40% in persons above 40 years of age and is more in rural areas) **(Habib et al., 2001 and Medhat et al., 2003)**.

Up to 70% of patients will have persistent infection after inoculation, making this disease a significant cause of morbidity and mortality. The severity of disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC) **(Asselah et al., 2009)**.

It has been estimated that HCV accounts for 27% of cirrhosis and 25% of HCC worldwide. HCV infection has likely been endemic in many populations for centuries **(Alter, 2007)**.

The major complication of chronic HCV infection is progressive hepatic fibrosis leading to cirrhosis, which develops in about 20% of those with chronic HCV **(Alter et al., 1999)**. The natural history of chronic HCV is variable, and progression of chronic liver disease is insidious in most patients. About one third of patients with chronic HCV develop hepatic cirrhosis 15 to 20 years after infection (rapid fibrotic progressors), one third develop cirrhosis 20 to 30 years after infection (intermediate fibrotic progressors), and one third develop it only after 30 years of HCV infection (slow fibrotic progressors) **(Alberti et al., 1999)**.

Cirrhosis is the most common non-neoplastic cause of death among hepatobiliary and digestive diseases **(Befeler and Bisceglie, 2002)**. Accurate diagnosis is crucial to the management of patients with chronic hepatitis B or chronic hepatitis C **(Friedman, 2003)**.

Liver biopsy has been used as the "gold standard" for assessment of hepatic fibrosis. It has been used for staging liver damage in chronic viral hepatitis and for decision analysis as to the need for treatment in patients with chronic hepatitis C. The limitations of biopsy, such as patient acceptability, sampling error, or diagnostic inaccuracy, and the remote risk of complications, have led clinical investigators to study alternative methods of staging chronic viral hepatitis **(Sandrin et al., 2003)**. Measurement of serum biological markers is the most widely used procedure for estimation of liver fibrosis (**Afdhal and Nunes, 2004**).

Hyaluronic acid is a glycosaminoglycan, produced by myofibroblasts, with a structural role in the extracellular matrix. It is degraded by liver sinusoidal cells, and its increased levels in serum may be linked to the endothelial dysfunction that occurs as fibrosis progresses. Several studies have shown a significant correlation between hyaluronic acid and fibrosis, especially in chronic hepatitis C patients **(Leroy et al., 2004)**.

Aim of work

The aim of this work is to evaluate the diagnostic value of Hyaluronic acid for detection of fibrosis and cirrhosis, in patients with chronic HCV infection and compare its results, as a non invasive marker, with the results of histopathological study.