Comparative Study Between serum Transforming Growth Factor β 1 (TGFβ1) level, serum Matrix Metalloproteinase-1 (MMP-1) level, Insulin Resistance and Liver Biopsy as predictors of Severity of Non Alcoholic Fatty Liver Disease (NAFLD) in Egyptian patients

Thesis

Submitted for partial fulfillment of M.D Degree

In Internal Medicine

By

Hosam Samir Ibrahim El Baz

M.B.B.Ch, M.Sc

Ain Shams University

Under supervision of

Professor Dr./ Hisham Ez El Din Said

Professor of Internal Medicine & Hepatology

Ain Shams University

Professor Dr./ Shadia Hussein Mabrouk

Professor of pathology

Ain Shams University

Assistant Prof./ Karim Yehia Ali Shahin

Assistant Professor of Clinical Pathology

Ain Shams University

Assistant Prof./ Amal Shawky Mohamed Bakir

Assistant Professor of Internal Medicine & Hepatology

Ain Shams University

Dr./ Sherif Sadek Taha Shabana

Lecturer of internal medicine & Hepatology

Ain Shams University

Faculty of Medicine

Ain Shams University

2008

Introduction:

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of elevated liver enzymes in adults and the most common cause of cryptogenic cirrhosis. NAFLD is deposition of fat in the liver of a non-alcoholic subject, a condition which may progress to end-stage liver disease. The spectrum of progression of NAFLD is similar to alcoholic liver disease, but is not caused by chronic alcohol consumption ***(Dabhi et al, 2008).***

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease NAFLD is probably the most common liver disease in many countries affecting 10% to 24% of the general population and its incidence is rising worldwide. Amongst the obese persons, the prevalence rises to 57 - 74% and 25 - 75% amongst obese diabetics. Accordingly, non-alcoholic steatohepatitis (NASH) can be considered as the 3rd commonest cause of liver disease after hepatitis C and alcohol abuse ***(Marchesini et al, 2005).***

The spectrum of NAFLD is wide and ranges from simple fat accumulation in hepatocytes (steatosis) without biochemical or histological evidence of inflammation or fibrosis, to fat accumulation plus necroinflammatory activity with or without fibrosis (steatohepatitis), Non Alcoholic Steatohepatitis (NASH) to the development of life - threating complications of cirrhosis and hepatocellular carcinoma ***(Madan et al, 2004).***

There is much scope for research to let us understand this disease and deal with it appropriately. Serum markers of fibrosis can offer an alternative or complimentary tool to identify patients with advanced disease. Understanding its pathogenesis, biochemical parameters, histological grading and staging, and its management, are vital issues today in clinical practice ***(Collantes et al, 2004).***

TGFβ1 mediates the transformation of quiescent Hepatic stellate cells (HSCs) into myofibroblast-like cells with an increased production of extracellular matrix proteins including type I collagen. TGFβ1 is secreted by activated Kupffer cells (KCs) and by Hepatic stellate cells (HSCs). Its plasma concentration is elevated in NASH patients compared to patients with steatosis and healthy subjects, suggesting that this cytokine is involved in the fibrogenesis in NASH ***(Hasegawa et al, 2001).*** Also, Matrix metalloproteinase 1 (MMP1) constitute another group of attractive candidate marker to be studied in NAFLD as they have been shown to affect the course of HCV virus infection by interfering with adequate matrix turn-over ***(Okamoto et al, 2005).***

Aim of the Work:

The aim of this study is to evaluate the significance of Serum level of Transforming Growth Factor β 1 (TGFβ1), serum matrix metalloproteinase (MMP-1) level and Insulin Resistance in comparison to liver biopsy in predicting the severity of Non Alcoholic Fatty Liver Disease (NAFLD) in Egyptian Patients.

Patients and methods:

This study will be conducted on 50 patients with documented NAFLD presenting to the hepatology clinic at Ain Shams University Hospital and 15 healthy individuals.

The candidates will be divided to 3 groups:

- Group A→ 25 NAFLD patients with mild (Grade 1) < 33% steatosis to moderate (Grade 2) 33–66% steatosis ***(Kleiner et al., 2005).***
- Group B→25 NAFLD patients with severe (Grade 3) > 66% steatosis ***(Kleiner et al, 2005).***
- Group C→15 healthy individuals with matched age and sex.

All candidates will be subjected to the following:

1. Full history taking.
2. Full clinical examination (including Body Mass Index (BMI), abdominal examination for hepatomegally).
3. Complete blood picture.
4. Complete liver function tests: Alanine animo transferasr (ALT), Aspartate amino transferase (AST), gamma glutamyl transferase (γGT), serum total bilirubin, serum direct bilirubin, serum albumin, Prothrombin time (P.T.) and International normalization ratio (INR).
5. Lipid Profile: including Total Cholesterol, Low Density Lipoprotein (LDL), serum Triglycerides.
6. Viral Markers: HBsAg, HBcAb (total), HCV Ab.

7. Autoimmune Markers: Anti Nucleic Acid Ab (ANA) - Anti Smooth muscle Ab- Anti Mitochondrial Ab (AMA), Liver Kidney Microsomal Ab (LKM).

8. Investigations to exclude metabolic liver disease: including serum iron, total iron binding capacity, serum ceruloplasmin, and 24 hour urinary cupper.

9. Serum level of Transforming Growth Factor β 1 (TGFβ1), serum matrix metalloproteinase-1 (MMP-1) level.

10. Insulin Resistance as assessed by the homeostasis model assessment-insulin resistance (HOMA-IR score): (Insulin0 x Glucose0)/ 22.5

    Units: insulin in μU/ml and fasting plasma glucose in mmol/L ***(Targher et al, 2005).***

11. Abdominal ultrasound: to detect sonographic findings of diffuse fatty change in the liver:

    (1) Diffuse hyperechoic echotexture (bright liver)
    (2) Increased liver echotexture compared with the kidneys
    (3) Vascular blurring.
    (4) Deep attenuation.

12. Ultrasound guided liver biopsy: the tissue will immediately be fixed in 10 % formal aldehyde. Parafin blocks and (H&E) stained sections of 5μm will be prepared and became ready for histopathological examination.

Exclusion criteria:

1) Other causes of chronic liver disease including alcoholic liver disease, viral hepatitis, autoimmune liver diseases and metabolic liver diseases.
2) Patients refusing to enroll in the study.

References:

Choudhury J and Sanyal A (2004): Clinical aspects of fatty liver disease. *Sem. Liv. Dis.;* 24, 349-362.

Collantes R, Ong JP and Younossi ZM (2004): Nonalcoholic fatty liver disease and the epidemic of obesity. *Cleve Clin J Med;* 71:657-64.

Dabhi AS, Brahmbhatt KJ, Pandya TP, Thorat PB and Shah MC (2008): Non-Alcoholic Fatty Liver Disease (NAFLD). *Indian Academy of Clinical Medicine*; 9(1):23-28.

Hasegawa T, Yoneda M, Nakamura K, Makino I and Terano A (2001): Plasma transforming growth factor-a1 level and efficacy of a-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. *Aliment Pharmacol Ther;* 15:1667–72.

Madan K, Batra Y and Panda SK (2004): Role of polymerase chain reaction and liver biopsy in the evaluation of patients with asymptomatic transaminitis; Implications in diagnostic approach. *J Gastroenterol Hepatol;* 19: 1291-1299.

Marchesini G, Marzocchi R, Agostini F and Bugianesi E (2005): Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol; 16:421-427.

Okamoto K, Mimura K, Murawaki Y and Yuasa I (2005): Association of functional gene polymorphisms of matrix metalloproteinase (MMP-1), MMP-3 and MMP-9 with the progression of chronic liver disease. *J Gastroenterol Hepatol*; 20: 1102-1108.

Targher G, Bertolini L, Scala L, Zoppini G, Zenari L and Falezza G (2005): Nonalcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in nondiabetic men. Role of visceral adipose tissue. *Diabet Med*.; 22:1354 – 1358.

List of abbreviations

2hr pp sugar	:	Two hours post prandial blood sugar
ACEI	:	Angiotensin-converting enzyme inhibitors.
ALD	:	Alcoholic liver disease.
ALT	:	Alanine amino transferase .
AMA	:	Anti mitochondrial antibody.
ANA	:	Anti nuclear antibody.
ANOVA	:	Analysis of variance.
ARB	:	Angiotensin-receptor blockers (ARB).
ASMA	:	Anti smooth muscle antibody.
AST	:	Aspartate amino transferase.
ATP	:	Adenosine triphosphate.
AUC	:	Area under the curve.
AUROC	:	Area under receiver-operator curve.
BMI	:	Body mass index.
CBC	:	Complete blood count.
CCL2	:	CC-chemokine ligand-2.
CHB	:	Chronic hepatitis B.
CHC	:	Chronic hepatitis C.
CI	:	Confidence interval.
CK-18	:	Cytokeratin-18.
CRP	:	C-reactive protein.
CT	:	Computerized tomography.
CYP 2E1	:	Cytochrome P-450 2E1.
DM	:	Diabetes mellitus.
ECM	:	Extracellular matrix.
ELF	:	European Liver Fibrosis Panel.
F.B.S	:	Fasting blood sugar.
FBS	:	Fasting blood sugar.
FFA	:	Free fatty acids.
GGT	:	Gamma-Glutamyltransferase.
GIP	:	Glucose-dependent insulinotropic polypeptide.
GLP-1	:	Glucagon-like peptide 1.

List of abbreviations(Cont.)

H1 MRS	:	Proton magnetic resonance spectroscopy.
HBs Ag	:	Hepatitis B surface antigen.
HBV	:	Hepatitis B virus.
HCC	:	Hepatocellular carcinoma.
HCV Ab	:	Hepatitis C antibodies.
HCV	:	Hepatitis C virus.
HDL	:	High density lipoprotein.
HIV	:	Human immunodeficiency virus.
HMG-CoA	:	3-Hydroxy-3-Methylglutaryl-coenzyme A.
HOMA-IR	:	Homeostasis Model Assessment of Insulin Resistance
HS	:	Highly significant.
HTGC	:	Hepatic triglyceride content (HTGC).
HTN	:	Hypertension.
HU	:	Hounsfield Unit.
IL	:	Interleukin.
INR	:	International normalized ratio.
IQR	:	Interquartile range.
IR	:	Insulin resistance.
ISHD	:	Ischemic heart disease.
kPa	:	Kilopascals.
LAGB	:	Laparoscopic adjusted gastric banding.
LCF	:	Liver cell failure.
LDL	:	Low density lipoprotein.
LFC	:	Liver fat content.
LLTs	:	Laboratory liver tests.
LS	:	Liver stiffness.
MHz	:	Mega hertz.
MMP-1	:	Matrix Metalloproteinase-1
MRE	:	Magnetic resonance elastography.
MRI	:	Magnetic resonance imaging.
MRS	:	Magnetic Resonance Spectroscopy.
NAFLD	:	Non-alcoholic fatty liver disease.

List of abbreviations(Cont.)

NAS	:	NAFLD activity score.
NASH	:	Non-alcoholic steatohepatitis.
NF-kappa β	:	Nuclear factor kappa β.
NIDDK	:	National institute of diabetes and digestive and kidney diseases.
NPV	:	Negative predictive value.
NS	:	Non significant.
OSI	:	Oxidative stress index.
PAS-d	:	Periodic- acid Shchiff with diastase.
PIII-NP	:	N-terminal peptide of procollagen-III.
PPAR-c	:	Peroxisome proliferator-activated receptorgamma.
PPV	:	Positive predictive value.
PT	:	Prothrombin time.
RAAS	:	Renin-angiotensin-aldosterone system.
ROS	:	Reactive oxygen species.
S	:	Significant.
SD	:	Standard deviation.
SPSS	:	Statistical package for the social sciences.
SR	:	Success rate.
T2DM	:	Type 2 diabetes mellitus.
TAR	:	Total plasma antioxidant response.
TBARS	:	Thiobarbituric acid reacting substance (TBARS).
TBT	:	Tributyltin .
TE	:	Transient elastography .
TG	:	Triglycerides.
TGF-beta	:	Tissue growth factor- beta.
TGFβ1	:	Transforming growth Factor β 1
TIMP-1	:	Tissue inhibitor of metalloproteinase-1.
TNF-α	:	Tumor necrosis factor-α.
TPN	:	Total parenteral nutrition.
UDCA	:	Ursodeoxycholic Acid.
ULN	:	Upper limit normal.

List of abbreviations(Cont.)

US	:	Ultrasound.
%	:	Percent.
- VE	:	Negative.
+ VE	:	Positive.

List of tables

Table	*Title*	*Page*
1	Conditions associated with NAFLD	10
2	Shows different methods for classification of severity of fatty liver in ultrasound	26
3	Characteristics of the ideal diagnostic marker of NAFLD	54
4	Human diseases and condition characterized by IR	85
5	Comparison between the 3 groups as regards the age using one-way analysis of variance	138
6	Comparison between the 3 groups as regards the gender using Chi-square test	139
7	Distribution of different clinical symptoms and signs in between groups I and II	140
8	Comparison between the 3 groups as regards B.M.I	141
9	Comparison between groups I and II as regards past history of D.M., HTN and ISHD	142
10	Comparison between the 3 groups as regards liver enzymes	144
11	Comparison between the 3 groups as regards other liver profile using ANOVA test	145

List of tables (Cont.)

Table	*Title*	*Page*
12	Comparison between the 3 groups as regards serum glucose levels and lipid profiles using ANOVA test	146
13	Comparison between the 3 groups as regards ultrasonographic finding	147
14	Comparison between the 3 groups as regards type of steatosis	148
15	Comparison between the 3 groups as regards necroinflamatory changes (grading) i.e. NAFLD Activity Score (NAS Score)	149
16	Comparison between the 3 groups as regards extent of fibrosis (staging)	151
17	Comparison between the 3 groups as regards mean of HOMA-IR	152
18	Comparison between the 3 groups as regards mean of serum MMP1	153
19	Comparison between the 3 groups as regards mean of serum TGFβ1	154
20	Comparison between histopathological findings according to mean HOMA among cases using ANOVA test	155
21	Correlations between histopathological parameters according to mean HOMA-IR using Pearson correlation (r)	155
22	On multivariate analysis between histopathological parameters according to mean HOMA-IR	156

List of tables (Cont.)

Table	*Title*	*Page*
23	Comparison between histopathological findings according to mean HOMA among cases using ANOVA test	156
24	Correlations between histopathological parameters according to mean MMP1 using Pearson correlation (r)	157
25	On multivariate analysis between histopathological parameters according to mean MMP1 (ng/ml)	157
26	Comparison between histopathological findings according to mean TGFβ1 among cases using ANOVA test	158
27	Correlations between histopathological parameters according to mean TGFβ1 using Pearson correlation (r)	158
28	On multivariate analysis between histopathological parameters according to mean TGFβ1 (ng/ml)	159
29	Diagnostic accuracy of HOMA-IR, serum MMP1 (ng/ml) and serum TGFβ1 (ng/ml) in quantifying extent of fibrosis (staging) in NAFLD patients	166

List of figures

Fig.	*Title*	*Page*
1	US of fatty liver	27
2	CT of fatty liver	29
3	Showing MRI picture of fatty liver	30
4	Synthesis and organization of latent complexes of transforming growth factor ß (TGF-ß)	103
5	SMAD Pathway	109
6	DAXX Pathway	111
7	Bar chart showing ccomparison between the 3 groups as regards the age	138
8	Bar chart showing comparison between the 3 groups as regards the gender	139
9	Bar chart showing ccomparison between the 3 groups as regards B.M.I.	141
10	Bar charts showing comparison between groups I & II as regards patient history of D.M., HTN and ISHD	143
11	Bar chart showing comparison between the 3 groups as regards ultrasonographic finding	147
12	Bar chart showing comparison between the 3 groups as regards type of steatosis	148
13	Bar chart showing comparison between the 3 groups as regards lobular inflammation, hepatocyte ballonimg and NAFLD Activity Score (NAS Score)	150
14	Bar chart showing comparison between the 3 groups as regards fibrosis staging	151